WEST Search History

DATE: Monday, July 15, 2002

Set Name side by side	**Query**	**Hit Count**	**Set Name** result set
	DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L5	L4 and l3	53	L5
L4	L2 and (analyzer$ or automat$)	85	L4
L3	L2 and (cytomet$3 or facs or (flow adj2 sort$3))	86	L3
L2	(erythroblast$ or nrbc or ((nucleated or nucle$2) adj2 (red blood cell$1 or erythro$5))) same (matur$ or stage$ or classif$7)	179	L2
L1	(erythroblast$ or nrbc or ((nucleated or nucle$2) adj2 (red blood cell$1 or erythro$5))) same (class?)	0	L1

END OF SEARCH HISTORY

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L5: Entry 20 of 53 File: USPT

DOCUMENT-IDENTIFIER: US 5891734 A
TITLE: Method for performing automated analysis

Abstract Text (1):
Provided are automated methods for distinguishing and differentiating cells in a whole blood sample. In one of the methods, a whole blood sample is provided. One or more tests to be performed on the whole blood sample is selected. The tests to be performed on the whole blood sample are correlated. A volume of the whole blood sample is aspirated into an automated instrument system which automatically performs conventional hematology analysis and fluorescent cytometry analysis on the whole blood sample. A first aliquot of the whole blood sample is dispensed into at least one sample receiving vessel. The first aliquot of the whole blood sample is mixed with a fluorescent reagent. The first aliquot of the whole blood sample mixed with fluorescent reagent is diluted and transported through a flow transducer system. The flow transducer system detects multi-angle light scatter and fluorescence from the first aliquot of the whole blood sample mixed with fluorescent reagent and counts and differentiates platelets or platelet clumps or both in the sample. Detecting and differentiation data for the one or more tests performed on the whole blood sample are stored. Results of the one or more tests performed on the whole blood sample are reported in a quantitative manner if so requested. The instrument system automatically performs all method steps without physically separating cells from the whole blood sample or an aliquot of the sample and results of a conventional hematology analysis may be utilized in at least reporting of results of the fluorescent cytometry testing.

Parent Case Text (2):
This is a continuation-in-part application of Ser. No. 08/482,678 filed Jun. 7, 1995 entitled METHOD AND APPARATUS FOR PERFORMING AUTOMATED ANALYSIS, now U.S. Pat. No. 5,656,499, which is a continuation-in-part application of Ser. No. 08/283,379 filed Aug. 1, 1994 entitled METHOD AND APPARATUS FOR PERFORMING AUTOMATED ANALYSIS, now abandoned. Both of the parent applications are assigned to the assignee of this application. The disclosures of both of the parent applications are incorporated herein in their entirety by this reference.

Brief Summary Text (2):
These embodiments relate in general to particle analysis. More particularly, they relate to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and flow cytometric techniques. These embodiments also relate to a multipurpose reagent system and a method for rapid analysis of a whole blood sample.

Brief Summary Text (6):
Peripheral blood also contains red cells of earlier maturation levels which are important diagnostic indicators. Two of these are reticulocytes and nucleated red blood cells.

Brief Summary Text (7):
At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an erythroblast. As the erythroblast matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to as nucleated red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in peripheral blood often

contribute to inaccurate enumeration of the white cell count, due in part to the presence of a nucleus which makes them difficult to distinguish from small white cells.

Brief Summary Text (8):
Reticulocytes are red cells at the maturation level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs as a result of an uncompensated increase in the rate of removal of erythrocytes from blood, or a decrease in the rate at which they are formed and released into blood. An increased reticulocyte patient count in an anemic patient indicates rapid erythroid turnover which suggests acute blood loss or hemolysis.

Brief Summary Text (16):
In the current state of the art of cell analysis, there are two technologies used for counting and classifying cells. These are generally known as "flow cytometry" and "image cytometry." The flow cytometry technology, which essentially consists of passing cells one at a time through a sensing zone of a flow cell, is preferred in clinical applications where patient test load is an important metric. This is mainly because it has at least an order of magnitude advantage in the number of cells that can be analyzed per second.

Brief Summary Text (17):
Instrumentation incorporating flow cytometry can be further subdivided into two methods which can be generally classified as "conventional hematology" and "fluorescence cytometry."

Brief Summary Text (18):
A primary distinction between the two methods is that conventional hematology generally distinguish cells by means of size and shape alone using primarily impedance and light scatter technologies, whereas fluorescence cytometry uses cell nucleic acid content and/or surface antigens in addition to size and shape in distinguishing cells. Therefore the fluorescence method may be used to subdivide the cell types into finer classifications.

Brief Summary Text (19):
A second distinction between the two methods is that conventional hematology gives results in absolute terms, whereas fluorescence cytometry results are in relative terms. Hematology analyzers deliver precise volumes and dilutions, and are thus able to measure absolute cell concentrations, or absolute counts of cell types per microliter of human blood. The fluorescence cytometry method gives only relative concentrations, or percentages of the various cell types.

Brief Summary Text (20):
A third distinction is that the hematology method is generally automated, whereas the fluorescence cytometric method as generally practiced today, is at best semi-automated, both in sample preparation, and in sample analysis. The fluorescence cytometry method is therefore significantly more labor intensive than the hematology method.

Brief Summary Text (22):
An example of an instrument for performing automated hematology measurements is the Cell-Dyn.RTM. 3000 instrument, which has been sold for several years by Sequoia-Turner, a predecessor in interest of Abbott Laboratories. The Cell-Dyn.RTM. 3000 instrument uses "impedance" measurements to count and size RBCs and PLTs, "absorption" measurements to determine the concentration of hemoglobin in RBCs (MCH), and "optical scatter" measurements to count and classify WBCs and the five part differential.

Brief Summary Text (23):
The Cell-Dyn.RTM. 3000 instrument automatically prepares blood samples, measures cell parameters and generates test results. The complete automation of sample preparation is such that no substantive operator intervention is required once the patient sample of whole blood has been presented to the analyzer. As mentioned previously, in order to assure accurate "patient counts" for the various cell classes, the Cell-Dyn.RTM. 3000 instrument provides precise sample volumes, reagent

volumes and dilution volumes. Patient counts are generally defined as the number of "events" per microliter of blood. The events may be RBCs, PLTS, WBCs, and classes or subclasses thereof.

Brief Summary Text (25):
In contrast, the fluorescence flow cytometer incorporates the principles of fluorescence cell analysis with light scatter. In general this requires that the cell be stained with an appropriate color dye, or that a fluorochrome label be attached to an antigen or antibody on the cell's surface thus indicating the occurrence of a specific antigen-antibody reaction.

Brief Summary Text (26):
In fluorescence flow cytometry, a suspension of previously stained or fluorescently labelled particles, typically cells in a blood or other biological fluid sample, is transported through a flowcell where the individual particles in the sample are illuminated with one or more focused light beams. One or more detectors detect the interaction between the light beam(s) and the labeled particles flowing through the flowcell. Commonly, some of the detectors are designed to measure fluorescent emissions, while other detectors measure scatter intensity or pulse duration. Thus, each particle that passes through the flowcell can be mapped into a feature space whose axes are the emission colors, light intensities, or other properties, i.e. scatter, measured by the detectors. Preferably, the different particles in the sample can be mapped into distinct and non-overlapping regions of the feature space, allowing each particle to be analyzed based on its mapping in the feature space. In this respect, flow cytometry differs from the conventional hematology instruments in that some of the feature space axis includes fluorescence emissions.

Brief Summary Text (27):
As noted above, lymphocyte subclasses are health determinants. Thus, it is desirable that these and other parameters be measured accurately. Although known hematology and fluorescent flow cytometry instruments have made significant advances in the ability to characterize blood cells, a problem still faced in this area is the difficulty in obtaining accurate patient count values for certain classes of cells.

Brief Summary Text (28):
An example of this problem is the CD4 cell patient count. Current analysis methods calculate the CD4 cell patient count from cell parameters measured on a hematology instrument and a separate fluorescence flow cytometry instrument. This calculation can provide up to 100% variability in absolute CD4 patient counts done on a single individual one week apart. See, e.g.: update, Testing In The Blood Bank, Volume 5, No. 2, pages 1 to 6, published 1991 by Ortho Diagnostics Systems, Inc.

Brief Summary Text (30):
The Lancet, Volume 340, Aug. 22, 1992, page 485 describes variation in CD4 count results when different analyzers are used. The variation appears to stem from different lymphocyte count results.

Brief Summary Text (33):
Laboratory Medicine, August 1983, Volume 14, No. 8, pages 509 to 514 discusses numerous spurious results and their causes in automated hematology analyzers.

Brief Summary Text (35):
One reason for variability in CD4 patient counts is manual sample preparation that cannot be controlled precisely and depends on operator proficiency. For example, a conventional procedure for determining a CD4 patient count starts with drawing two tubes of blood from a patient. One tube is analyzed on a hematology instrument which generates several measured and/or calculated parameters for the blood sample, including a total lymphocyte patient count, a lymphocyte percentage and a total WBC patient count. The second tube of blood is analyzed on a fluorescence flow cytometry instrument. The sample preparation steps for the flow cytometry tests are labor intensive and operator dependent. These steps do not readily lend themselves to automation and precision.

Brief Summary Text (36):
To prepare the sample for the flow cytometry instrument, the operator manually

pipettes a volume of blood from the sample tube into an analysis tube. A volume of the desired fluorochrome labeled monoclonal antibody is added. The sample/antibody mixture is then incubated for a predetermined time at a predetermined temperature to allow antibody/antigen bindings to take place. After incubation, the operator adds a volume of RBC lyse to destroy the RBCs in the sample. Timing is important during the lysing stage. If the operator does not allow the lyse reaction to continue long enough, RBCs may remain in the sample and distort the measurements. If the operator allows the lyse reaction to continue for too long, the lyse may attack the WBCs.

Brief Summary Text (37):
After determining that the lyse reaction is complete, the operator centrifuges and washes the sample to remove any debris left over from lysed RBCs. The centrifuge/wash step may be performed several times until the operator is satisfied that the sample is sufficiently clean. Debris, red cell "stroma" can interfere with the detection processes of the typical flow cytometer. The sample now contains WBCs with antibodies bound to cells bearing the complementary surface antigens. The operator re-suspends the sample in a volume of fixative, and then passes the sample through the fluorescence flow cytometry instrument.

Brief Summary Text (38):
The fluorescence flow cytometry instrument generates only percentage values for lymphocyte subsets. This is at least partially due to the fact that the numerous manual dilutions and volume reductions performed during the sample preparation steps do not allow the isolation of a precise measurement volume. Thus, the fluorescence flow cytometry instrument identifies the CD4 positive helper-T cells as the percentage of lymphocytes which are both positive for CD3 (T cell marker), and positive for CD4 (helper-T marker).

Brief Summary Text (40):
The lymph count and the WBC patient count are taken from the hematology instrument, while the "% helper-T cells in lymph" value is taken from the fluorescence instrument after a correction factor is applied based on the flow cytometer mapping of scatter and fluorescence.

Brief Summary Text (43):
Previous attempts to automate sample preparation in fluorescence cytometry testing have only been partially successful. Such systems still require the operator to perform sample preparation steps such as separating lymphocytes from other peripheral blood cells by density gradient centrifugation, and/or lysing red cells, removing red cell ghosts and cell debris by centrifugation, or preserving the morphology of the remaining white cells by suspending the white cells in an isotonic saline solution containing appropriate fixatives. These operations generally require the operator to manually alter the volume of the sample, thus compromising sample volume precision which can be achieved with automated mechanical volume dispensers.

Brief Summary Text (45):
In leukocyte analyses, it is desirable that all of the RBCs be lysed. Because RBCs outnumber WBCs by about 700 to 1, a small number of unlysed red cells may significantly distort white cell patient counts. Some reagents used to lyse red cells require too lengthy an incubation period to be practical in an automated clinical analyzer. For example, the Tris buffered ammonium chloride solution recommended by K. A. Murihead in Clinical Cytometry, Ann. N.Y. Acd. Sci., vol. 468, pp. 113-127 (1986) takes about 5 to 10 minutes to lyse red cells, which may be impractical for automation.

Brief Summary Text (46):
Furthermore, incomplete hemolysis with certain lytic reagents may result in red cell stroma that retain sufficient hemoglobin or particulate matter to generate high background patient counts in automated clinical electro-optical systems. When this occurs, it is usually necessary to remove the WBCs to be analyzed from the red cell stroma by centrifugation, a procedure that is a limiting factor when adapting a reagent system for automation.

Brief Summary Text (48):
The earliest stage of RBC, the nucleated red cell, NRBC, when found in the

peripheral blood on conventional hematology analyzers can be confused for a small lymphocyte, since the lysis will not destroy the nucleus of the NRBC. Because of the ratio of RBCs to WBCs, even a relatively small percentage of NRBCs can lead to substantial error in the WBC and lymphocyte count. This may be troublesome in neonate or pediatric samples, in which the presence of NRBCs in peripheral blood is a normal condition. For this reason, the laboratory may do manual slide inspections on some of these samples. Conventional hematology analyzers are only able to flag these samples by noting the spreading out of the usual lymphocyte scatter cluster. The manual inspection results in a count of the number of NRBCs per 100 nucleated cells. This percentage is then used to correct the analyzer WBC count as follows

Brief Summary Text (49):
Clearly the need exists for an accurate automated count of NRBCs.

Brief Summary Text (52):
Platelet counts are also a health determinant. Some hematology analyzers, such as the CELL-DYN.RTM. 3000 and others mentioned earlier, count platelets by an impedance method. This method has limitations when the platelet count is reduced, such as about less than or equal to about 50,000 per .mu.l. These limitations may include lack of precision due to the relatively few platelets counted, inaccurate results due to the only one dimensional measurement provided by the impedance transducer, etc. Further, because of the one dimensional measurement, the analysis may confuse other cell fragments with platelets as they pass through the impedance sensing chamber. Thus, improvements in platelet analysis are also desired.

Brief Summary Text (54):
Provided are automated methods for distinguishing and differentiating cells in a whole blood sample. In one of the methods, a whole blood sample is provided. One or more tests to be performed on the whole blood sample is selected. The tests to be performed on the whole blood sample are correlated. A volume of the whole blood sample is aspirated into an automated instrument system which automatically performs conventional hematology analysis and fluorescent cytometry analysis on the whole blood sample. A first aliquot of the whole blood sample is dispensed into at least one sample receiving vessel. The first aliquot of the whole blood sample is mixed with a fluorescent reagent. The first aliquot of the whole blood sample mixed with fluorescent reagent is diluted and transported through a flow transducer system. The flow transducer system detects multi-angle light scatter and fluorescence from the first aliquot of the whole blood sample mixed with fluorescent reagent and counts and differentiates platelets or platelet clumps or both in the sample. Detecting and differentiation data for the one or more tests performed on the whole blood sample are stored. Results of the one or more tests performed on the whole blood sample are reported in a quantitative manner if so requested. The instrument system automatically performs all method steps without physically separating cells from the whole blood sample or an aliquot of the sample and results of a conventional hematology analysis may be utilized in at least reporting of results of the fluorescent cytometry testing.

Brief Summary Text (55):
In another method, a whole blood sample is provided. A series of two or more tests to be performed on the whole blood sample is selected. The tests to be performed on the whole blood sample are correlated. A first volume of the whole blood sample is aspirated into an automated instrument system which performs conventional hematology analysis and fluorescent cytometry analysis on the whole blood sample. Aliquots of the whole blood sample are dispensed into at least three sample receiving vessels. A first aliquot of the whole blood sample is diluted with a diluent reagent. A second aliquot of the whole blood sample is lysed with a lysing reagent. A third aliquot of the whole blood sample is mixed with a fluorescent reagent. The first aliquot of diluted whole blood sample is transported through a flow transducer. The instrument flow transducer detects and counts red blood cells and platelets in the first aliquot of diluted whole blood sample. The second aliquot of lysed whole blood sample is transported through a flow transducer system. The flow transducer system detects multi-angle light scatter from the second aliquot of lysed whole blood sample and counts and differentiates white blood cells in the second aliquot of whole blood sample. The flow transducer system detects multi-angle light scatter and fluorescence from the second aliquot of lysed whole blood sample or the first

aliquot of diluted whole blood sample and counts and differentiates nucleated red blood cells or reticulocytes or both therein. The third aliquot of the whole blood sample is transported through a flow transducer system. The flow transducer system detects multi-angle light scatter and fluorescence from the third aliquot of whole blood sample and counts and differentiates platelets or platelet clumps or both therein. The instrument stores detecting and differentiating data for multiple tests performed on the whole blood sample. The instrument reports results of each of the multiple tests performed on the whole blood sample in a quantitative manner if so requested. The instrument system automatically performs all method steps without physically separating cells from the whole blood sample or an aliquot thereof and results of the conventional hematology analysis may be utilized in at least reporting of results of fluorescent cytometry testing.

Brief Summary Paragraph Equation (3):
Corrected WBC count=Analyzer count (1-manual NRBC percentage/100)

Drawing Description Text (18):
FIG. 13 is a timing diagram illustrating one embodiment of an integrated, automated, hematology/immunology sample processing method of the cell analysis system shown in FIG. 1;

Drawing Description Text (30):
FIG. 25 is a block diagram illustrating one embodiment of the analyzer module of the cell analysis system shown in FIG. 1;

Drawing Description Text (32):
FIG. 27 is a block diagram illustrating further details of the analyzer module shown in FIG. 25;

Detailed Description Text (2):
Embodiments of the present invention comprise an analytical instrument system and a method for analyzing fluid samples. Generally, one such automated instrument system includes a conventional hematology analyzer fully integrated with a controller and a fluorescent cytometer. The instrument system is able to distinguish and classify cells, whereby the data collected by the hematology analyzer is automatedly utilized by the fluorescent cytometer to process samples, analyze sample and classify cells within the sample and report quantitative as well as qualitative results.

Detailed Description Text (3):
The automated instrument system herein disclosed combines or integrates conventional hematology with fluorescent cytometry on a single analyzer platform. Heretofore, this approach has not been possible. Both methods benefit by this unique combination. Fluorescence information is improved by total automation and absolute concentrations. The hematology information is enhanced by adding fluorescence cytometry to the technology of colorimetry, impedance, and multi-angle light scatter, thereby enabling superior hematology and total automation of tests which currently are done either manually, or on separate and distinct analyzers.

Detailed Description Text (4):
For the sake of this disclosure, automation is distinguished in that an operator does not need to intervene in the sample preparation process or analysis of the sample, once the sample, i.e., whole blood, urine, saliva etc., is presented to the instrument. Additionally, all sample handling, processing and analyzing steps and functions are carried out automatedly by the instrument based upon the tests selected by the operator. All data and other information pertaining to each initial test sample is monitored, collected, and processed by the instrument controller.

Detailed Description Text (5):
The embodiments of the invention generally comprise an automated hematology analyzer and a flow cytometry analyzer integrated with a controller which monitors and controls the analyzers, collects data from the analyzers and reports a result. Illustrating by example, integration of the analyzers with a controller allows an operator to input data about a whole blood sample into the controller. The operator selects a series of tests to be performed on the sample, generally whole blood, with the aid of the controller. The operator presents the whole blood sample to the

integrated analyzers at a centralized sample handling, or processing area. The controller activates the analyzers, allowing the analyzers to automatedly perform analyses on the whole blood sample under the direction of the controller. The controller utilizes data obtained from the analyzers to formulate a result. The controller reports the result to the operator. It is to be noted that no operator action is needed after the whole blood sample is presented to the integrated analyzers. Because the whole blood sample preparation is entirely automated, in a preferred embodiment, conventional hematology tests are done first with the incubated sample tests to follow. Because the analyzers are integrated with the controller, the controller obtains data from both the hematology analyzer and the flow cytometry analyzer. Thus, the controller is able to report a combined patient blood analysis to the operator, In addition absolute concentrations are reportable because of the precision and repeatability of automated dilution, cell preparation and analysis. Human error has all be been eliminated because the instrument system is the only thing to touch the sample once the operator has programmed the instrument and placed the sample on-board.

Detailed Description Text (8):
FIG. 1 is a block diagram of a cell analysis system 60. The system 60 includes an analyzer module 64, a data station module 68, and a pneumatic unit 72. The analyzer module 64 is operatively connected to the data station module 68 by a serial data link 76 implementing a HDLC (high level data link) protocol. The pneumatic unit 72 is operatively connected to the analyzer module 64 by a serial data link 84 and a network of tubing 80.

Detailed Description Text (9):
The analyzer module 64 aspirates samples, diluent and reagents, dilutes samples, measures and collects data, transmits measured data to the data station module 68, manages reagents, and disposes of waste. An exemplary analyzer module 64 includes its own power supply, impedance transducer, HGB transducer, optical flowcell/transducer (light scattering and fluorescence), optical detectors, electronics, reagent reservoirs, fluidics system, integrated and fully automated sample processor for both hematology and fluorescent cytometry tests, and any necessary incubation and/or cooling systems. An exemplary analyzer module includes a Motorola 68302-type microcomputer that controls mechanical components of the analyzer 64 and executes the analyzer's flow sequences.

Detailed Description Text (10):
The pneumatic unit 72 houses pneumatic sources for moving fluids through the analyzer module 64. The pneumatic unit 72 receives instructions from the analyzer module 64 via that serial data link 84.

Detailed Description Text (11):
The data station module 68 provides general controls to the analyzer module 64, converts measured data into meaningful test results, stores measured data and test results, prints reports, and provides bi-directional communication with an off-line host computer (not shown). An exemplary data station module 68 includes an 80386 or 80486-type microcomputer, color display, 31/2 inch disk drive, at least 540 megabyte hard desk, PC-style keyboard, a pointing device, and LAN connections. The data station 68 includes memory, such as a RAM, a ROM, an EPROM, a SRAM and the like, having sufficient software algorithms to manipulate measured data, calculate parameters, and display results in a variety of formats, including histograms, scattergrams, and other multidimensional plots.

Detailed Description Text (17):
The multipurpose reagent system is utilized in the automated determination of differential white cell patient counts, nucleated red blood cells, and lymphocyte immunophenotyping. A method for the rapid analysis of nucleated peripheral whole blood cells includes the following steps: mixing the described multipurpose reagent system with an anticoagulated whole blood sample (whereby the blood is diluted 10 to 100 fold), mixing the diluent-blood mixture at temperatures from about 25.degree. C. to 46.degree. C. for at least about 10 seconds, and analyzing the nucleated peripheral blood cells with the automated cell analysis system of the present invention.

Detailed Description Text (26):
A further embodiment of the multipurpose reagent system allows for the quantitative analysis of lymphocyte subpopulations. Lymphocyte subclassification is achieved by mixing fluorochrome-conjugated monoclonal antibodies (directed to specific lymphocyte surface antigens) with whole blood samples before adding the multipurpose reagent system, or blood diluent. The concentration of labeled antibody fractions added to a blood sample depends upon the individual antibody preparation, but is commonly about one-half to one-tenth of the volume of the blood for commercial antibody preparations. After the reagent system is added and the red cells are lysed, the lymphocyte-antibody reaction products can be analyzed on an automated flow cytometric system. There is no need to "separate" the lymphocytes from the lysed cells by centrifugation and washing as is common in the art.

Detailed Description Text (29):
The cell analysis system 60 utilizes an automated method for simultaneous analysis of WBC/Diff and NRBC in a whole blood sample using a unique triple triggering method with lyse reagent, such as the rapid lyse reagent system described above. This method, claimed in U.S. patent application Ser. No. 08/356,932, entitled "Method For Rapid And Simultaneous Analysis Of Nucleated Red Blood Cells", and filed on Dec. 15, 1994, now U.S. Pat. No. 5,559,037 enables the accurate NRBC counts and WBC/Diff data, simultaneously from a whole blood sample containing NRBC. The entire contents of U.S. Ser. No. 08/356,932 is hereby incorporated by reference.

Detailed Description Text (32):
The triple trigger method is unique in that the simultaneous analysis of WBC/Diff/NRBC can be carried out automatically, accurately, and rapidly without interference from other cellular debris such as RNA from lysed reticulocytes, Howell Jolly Bodies, reticulated platelets, giant platelets, DNA from WBC and Megakaryocytic fragments, parasites, and RBC fragments.

Detailed Description Text (49):
No matter what the formulation of the lyse utilized with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital nuclear stain which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the herein referenced analyzer, the lysis chemistry will be configured such that the refractive index matches that of a sheath solution to substantially less than 0.1%.

Detailed Description Text (53):
When cells, thus triggered, pass through the aforementioned illuminated volume, pulses are generated at detectors 380, 400, 401 and 404. The amplitudes of these pulses are then filtered, amplified, digitized, and stored in list mode in the corresponding five dimensional feature space of ALL, IAS, FL3, PSS (polarized side scatter), and DSS (depolarized side scatter). The normal counting time through flowcell 170 is 10 seconds. At the flow rate and dilution ratio described above, with a normal patient WBC count of 7000 cells per microliter of blood volume, the resulting event count rate would be 5000. In low count samples, this counting time can be automatically extended in order to improve the statistics of the measurement. At the conclusion of the measurement time, the sample stream is piped to waste, and probe is cleaned and dried and prepared to process a subsequent sample.

Detailed Description Text (83):
The method allows the enumeration of reticulocytes from a whole blood sample while simultaneously differentiating a separate aliquot of the sample to obtain a complete blood cell ("CBC") analysis. This method comprises, directing one or more aliquots of the sample to various positions within an automated analyzer for analysis and differentiation, while a reticulocyte aliquot of the sample is combined with a staining reagent.

Detailed Description Text (84):
The combined reagent/reticulocyte aliquot is then directed to an optical flow cell 170 of the automated analyzer 60. Thereafter the reagent/reticulocyte aliquot is passed through an illuminated sensing zone 300 essentially one cell at a time to cause fluorescence and scattered light events. These events are detected and the number of reticulocytes present in said sample are determined therefrom.

Detailed Description Text (100):
In order to analyze a whole blood sample for the percentage as well as the absolute counts of reticulocytes on the multi-parameter hematology analyzer described above, about 18.75 .mu.l of a whole blood sample is deposited by means of a sample aspiration probe into the RBC cup 134 which contains about 7856 .mu.l of a diluent/sheath solution (an isotonic saline) and the fluids are mixed. The diluted sample is then transported to a sheathed impedance aperture 174 to electronically determine the absolute RBC counts of the sample. In the mean time, about 200 .mu.l of the diluted sample is transferred into Retic cup 136 which contains 600 .mu.l of the disclosed reagent, where it is mixed. The prepared (mixed) sample is then transported to the sheathed optical flow cell 170 for detection. The measurement process begins as the cell stream passes through the flow cell essentially one cell at a time, in a laminar flowing sample stream surrounded by a diluent-sheath solution, disclosed hereinafter.

Detailed Description Text (126):
Another embodiment provides a method for enumeration of reticulocytes in a whole blood sample using flow cytometry wherein an aqueous staining solution of a 2,2'-dye which is capable of staining RNA-containing material is placed in contact with an RNA-containing material for a period of time adequate to enable the staining solution dye equilibrate with the RNA-containing material. The stained sample is then directed through the optical sensing zone of a flow cytometry instrument and illuminated once within the optical sensing zone with an incident light beam. The fluorescence of the reticulocytes in sample solution are then measured as they pass through the optical sensing zone.

Detailed Description Text (130):
The 2,2'-dyes can be used in any conventional assay technique which requires the staining of reticulocytes with a fluorescent marker. In particular, these dyes can be used in any assay for which thiazole orange is currently recommended, such as reticulocyte detection and enumeration in an argon ion laser flow cytometer.

Detailed Description Text (131):
When these class of dyes are utilized to detect and differentiate reticulocytes an incubation site and associated temperature controls and sample handlers must be provided for within the instrument and operatively connected to the analyzer to maintain the automation of the inventive instrument system disclosed herein.

Detailed Description Text (134):
A cyanide-free reagent must be able to quickly lyse the erythrocytes and rapidly complex with the hemoglobin so that a detectable chromogenic structure is formed for detection and measurement. The disclosed reagent is stable for many weeks and is particularly advantageous because the resulting chromogen appears to be free of interference from other blood components and can be measured at wavelengths in the spectral range of automated hematology instruments already in the field. For comparison purposes, the cyan met hemoglobin method typically measures absorbance at 540 nm. A reddish brown chromogen can be formed according to the present invention which has an absorption maximum at about 544 nm.

Detailed Description Text (143):
8. Analyzer Module

Detailed Description Text (144):
A. Automated Sample Transport

Detailed Description Text (145):
The analyzer 64 may be provided with an autoloader (not shown) for automatically transporting sample tubes to the analyzer 64 for processing. Such an autoloader may include a holder which retains up to about 100 sample tubes of various sizes. A presenter which sequentially presents the sample tubes to the analyzer 64 for aspiration is operatively connected with the autoloader. A mixer which mixes the sample just before sample aspiration may also operatively associated with the autoloader. A bar code reader for reading the bar code label on each tube can also operatively be associated with the autoloader and operatively connected to the

system controller to input sample information into the system controller.

Detailed Description Text (146):
B. Automated Sample Processing and Measurement

Detailed Description Text (147):
FIG. 3 illustrates a top view of one embodiment of an automated sample processing area 110 for use in the cell analysis system 60 shown in FIG. 1. The processing area 110 is part of the analyzer 64 portion of the cell analysis system 60. The processing area 110 includes a sample cup area 114 and an incubation area 118.

Detailed Description Text (149):
The reagent modules 122 include wells 128 for holding a volume of antibody reagent. In the illustrated embodiment, each reagent module 122 has a housing with a reagent well 128, preferably six in number, packaged with a particular panel of reagents. The reagents in each panel are selected so that, for the tests associated with each panel, an approximately equal amount of reagent is used from each well 128. If less than six reagents are required for the test associated with the panel, the excess wells 128 are covered by a plug (not shown). Each reagent module 122 is also fitted with a memory, such as a non-volatile RAM and the like, to store module ID and usage information. The reagent modules 122 are preferably keyed so that they may be seated in an opening (not shown), located in the thermostated block 120, in a predefined orientation. This allows the central processing unit (CPU) of the analyzer 64 to store the location and, from the usage information, the volume of the contents of each well 128 in each reagent module 122.

Detailed Description Text (185):
FIG. 5 further illustrates the analyzer's sample processing. As shown in FIG. 5, several of the sample processing cups 132, 134, 136, 138, 140 and 142 are connected to the flowcells/transducers 170, 174, 178 via a network of transport tubing 182. The RBC cup 134, RETIC cup 136, and WBC cup 138 are each in fluid communication with the impedance transducer 174 and the optical flowcell 170. The HGB cup 142 is in fluid communication with the HGB transducer 178.

Detailed Description Text (187):
In the disclosed embodiment, the analyzer module 64 is supplied with diluent, monoclonal antibody (MAb) reagents if necessary, several lysing reagents, and reticulocyte stain. The diluent, lysing reagents, and reticulocyte stain are supplied through reservoirs 192 and 196 (shown in FIGS. 7, 8 and 9) coupled to the analyzer 64. The reservoirs 192 for diluent and lysing reagents are also coupled to bulk storage containers 193. When the flow script request the filling of a reservoir, the level sensing switch (not shown) in the reservoirs 192 checks for a full condition in the reservoir, and if the instrument controller determines that the reservoir can tolerate the filling sequence at this time, a pneumatic control line 189 switches from applying a positive pressure to applying a vacuum of about 15 inches of mercury. This vacuum causes fluid to flow from the bulk storage container 193 into the reservoir 192 until the level sensing switch senses that the reservoir 192 is full, at which time the pneumatic control line 189 returns to a positive pressure and fluid flow from the bulk storage container 193 to the reservoir 192 ceases. The Mab reagents can be supplied by disposable, pre-packaged reagent modules 122 (shown in FIGS. 3 and 4).

Detailed Description Text (188):
The analyzer 64 is provided with fluid sensors (not shown) for determining when one of the bulk containers is empty. These sensors detect air bubbles drawn into the tubing between the bulk storage containers 193 and the reservoirs 192. The analyzer 64 informs the data station module 68 which, in turn, signals the operator about the empty container. The operator can then replace the empty container with a full one and indicate via the user interface to the data station 68 that the container has been replaced. Until the container is replaced, the analyzer 64 will not aspirate additional samples from the sample tubes, although processing of samples already begun will continue with the sufficient reagent remaining in the reservoirs.

Detailed Description Text (189):
The aspiration and dispensation by the aspiration probe 156 and the incubation probe

160 are effected by a series of piston pumps 190. FIGS. 7 and 8 illustrate how the aspiration probe 156 and incubation probe 160 are connected to piston pumps 190 and the reagent reservoirs 192. The volume and flow rate of these fluid transfers are controlled by the analyzer 64 and the data station 198.

Detailed Description Text (195):
FIG. 10a illustrates bulk transfer of sample from a sample cup 216 to the proximity of impedance transducer 174 via pump 220. FIG. 10b illustrates metered delivery of the sample by the RBC delivery syringe 204 to the impedance transducer 174. The sample cup 216 is connected to the RBC syringe 204, the impedance transducer 174 and a peristaltic pump 220 by tubing 182. A first valve 210 is placed in the tubing 182 downstream of the sample cup 216, and a second valve 212 is placed in the tubing 182 upstream of the peristaltic pump 220. The flow rate and general operation of the RBC syringe 204 are controlled automatically by the analyzer's electronics and software.

Detailed Description Text (241):
A plan view of the optics bench 350 is shown in FIG. 19. The optics bench 350 is mounted on the analyzer module 64 and includes a laser light source 352, mirrors 354, 356, lenses 358, 360, a flowcell 170 (fused-silica in an exemplary embodiment), and several detectors 400, 402, 404. The laser beam 368 is directed by a rear mirror 354, a front mirror 356, a beam adjuster 370, shaped and focussed by a pair of cylindrical lenses 358 and a laser focusing lens 360.

Detailed Description Text (259):
In a preferred embodiment of the cell analysis system 60, the pneumatic unit 72 is a separate unit having a dedicated power supply. This construction reduces weight, size and power consumption of the analyzer module 64 and data station module 68.

Detailed Description Text (261):
The vacuum pressures are controlled by the analyzer software present in a suitable memory, such as a RAM, a ROM, an EPROM, a SRAM and the like.

Detailed Description Text (264):
The data station 68 of the cell analysis system 60 has memories and other devices which apply algorithms for various cellular analyses. These algorithms are used to analyze clusters of data points generated by the analysis module 64 to yield information of clinical relevance. The disclosed integrated hematology/immunology instrument provides a single platform on which such software may be implemented, thereby providing an instrument that not only automates hematology and immunology sample processing and measurement, but also automates data analysis.

Detailed Description Text (267):
Electronic systems are found in the analyzer module 64, data station module 68, and pneumatic unit 72. The analyzer 64 provides the hardware platform for data acquisition and fluidics and motion control. In an exemplary embodiment, the data station 68 is a general purpose computer that serves as a user interface and processes, displays and stores the acquired data. The pneumatic unit 72 controls the vacuum and pressure sources.

Detailed Description Text (268):
In a preferred embodiment, the three modules are physically separate, and each unit is powered from a separate AC outlet The data station 68 and the pneumatic unit 72 communicate with the analyzer 64 through independent serial communication channels 76, 84.

Detailed Description Text (269):
FIG. 25 is a block diagram illustrating some electronic hardware components of the analyzer 64. These components include a central processing module 500 (CPM), a data acquisition subsystem 502, and a motion control subsystem 504. The CPM 500 controls the data acquisition subsystem 502, the motion control subsystem 504, and communication functions.

Detailed Description Text (315):
The signal processing module 514 uses a 16-bit counter (not shown) to generate a

time stamp with an approximately 0.5 ms resolution. The time stamp value is stored with the data from each automatic sequence iteration which resulted in valid data acquired in the ADC module 516.

Detailed Description Text (317):
FIG. 27 is a block diagram illustrating an exemplary embodiment of the motion control subsystem 504. The flow sequences and automated sample processing operations of the analyzer 64 are controlled through the motion control subsystem 504.

Detailed Description Text (318):
As illustrated, the motion control subsystem 504 includes a motor processing module 520 (MPM), a valve control module 522 (VCM), a fluid sensor module 524 (FSM), and a digital input module 526 (DIM). The MPMs 520 communicate with the CPM 500 through two independent serial links 530, 532 (500 KB), and each MPM 520 preferably controls up to 12 stepper motors 534. The VCMs 522 control all valves in the analyzer 64. The DIMs 526 monitor all digital inputs (switches, optical sensors, and magnetic sensors). The FSM 524 monitors all fluid sensors.

Detailed Description Text (321):
Software controls the major operations of the cell analysis system 60, including the analyzer flow sequences, the timing and sequence of events gathering data, and converting measured data into meaningful results. The software is resident on suitable memories, such as RAM's, ROM's, EPROM's, SRAM's and the like, found in the system 60. The software components are preferably partitioned into the six domains (represented by circles) shown in FIG. 2.

Detailed Description Text (323):
The data station operating software 92 controls sample processing, data management, security, communications with the analyzer module and laboratory information systems (LIS), and generation of printed outputs.

Detailed Description Text (325):
The analyzer operating software (AOS) 98 controls the analyzer module's electronics (hardware), data collection, and communications to the data station module. The timing and scheduling of all analyzer activities, including the analyzer flow sequences, is also controlled by the AOS 98.

Detailed Description Text (326):
The flow sequence (FSQ) software 100 controls the mechanical components responsible for moving fluids through the analyzer module 64, including the execution of automated sample processing protocols and integrated hematology and immunology testing.

Detailed Description Text (327):
The firmware 102 includes a network of EPROM resident device controllers for various hardware modules of the analyzer 64 and pneumatic unit 72.

Detailed Description Text (328):
The operator interface (OI), data station operating software (DSOS), and algorithms use the data station module 68 as their platform. The AOS 98, FSQ software 100, and firmware 102 reside in and use the analyzer module 64 as their platform. The preferred software is a multitasking, multithreaded application.

Detailed Description Text (329):
The AOS 98 resides in the CPM 500 and is the main controller of the detailed operation of the analyzer 64. It communicates with several slave microcontrollers responsible for stepper motor timing analog-digital conversion, vacuum/pressure closed loop monitor/control, valve control, and digital sensor inputs. In addition, it is responsible for data, status and control communication with the data station 68 to which it is connected. The AOS 98 is preferably executed on a Motorola 68302 CPU chip. Its firmware is stored in external EPROM(s), and the downloaded AOS and flow sequences are stored in on-board RAM. An embodiment of AOS operation is shown in FIGS. 29 and 30.

Detailed Description Text (331):

Each flow sequence requires tasks of multiple analyzer components in accordance with a schedule. FIG. 13 is a timing diagram of an exemplary flow sequence for integrating and automating hematology and immunology sample preparation and measurement on a single unit. The upper-most horizontal axis, as viewed, represents time in seconds, and the left-most vertical axis lists sample processing and measurement components of the analyzer 64. The grids of the diagram describe the activities of the analyzer components. Each of the components listed along the left vertical axis in FIG. 13 performs a specific set of tasks in the flow sequence. When a component has completed its task, it begins to look for its next instruction without waiting for downstream components to finish work on the current sample.

Detailed Description Text (334):
To commence a flow sequence, the AOS 98 determines that a sample is available for aspiration. This is based either on operator activation of a pushbutton or a command from an autoloader mechanism. All the information known by the analyzer 64 about the sample is sent to the data station 68. The data station 68 responds with information about the required measurements to be performed on the sample. Based upon this response, and in conjunction with the state of the analyzer 64 (i.e. reagents, incubations, flow sequence aspiration enable/disable flags), the AOS determines whether or not to proceed with sample aspiration. Whether or not an aspiration occurs, the AOS informs the data station 68 of the status of the sample.

Detailed Description Text (337):
Upon data station request, the AOS supplies the current incubation status of all sites in the analyzer 64. This information includes incubation time, site status (clean/dirty) and site usage counts.

Detailed Description Text (340):
The flow sequence interpreter allows flow sequences to initiate event count and data collection intervals. Data generated during the data collection interval is automatically sent to the data station 68 by the AOS. The data sent to the data station 68 preferably includes at least the sample identifier, hardware counters, list mode data, and incubation time (if any). Count types preferably include:

Detailed Description Text (344):
The AOS allows the analyzer 64 to overlap counting activity on the flowcells/transducers 170, 174, 178. Thus, multiplexing and piplining the analyzer activity maximizes instrument throughput.

Detailed Description Text (345):
The analyzer 64 may be connected to external containers for waste (not shown) or bulk reagent storage (193). AOS monitors sensors that detect when the waste container becomes full or a bulk reagent storage container 193 becomes empty. Further aspiration of samples is inhibited by the AOS 98 until the condition is remedied.

Detailed Description Text (355):
The antibody reagent modules 122 are read as part of normal analyzer initialization. Thereafter, any operation that affects the status of the module 122 is recorded in the module's memory.

Detailed Description Text (356):
The AOS 98 communicates with the motor processor modules 520 which are responsible for controlling the analyzer stepper motors 534. The AOS resets the motor processor modules 520 at initialization. The AOS keeps track of the position of each motor in the analyzer 64 and verifies this information with the controlling motor processor module 520. Position discrepancies are reported to the data station 68.

Detailed Description Text (357):
Upon successful completion of power-on self tests, the analyzer 64 accepts AOS operating software downloaded from the data station 68. At the completion of the software download, a start address is supplied from the data station 68 specifying the address at which to begin execution.

Detailed Description Text (361):

The automated sample processing protocol of the cell analysis system 60 can be considered in three phases--sample preparation, sample measurement, and sample analysis. The particular protocol for each of these phases is test dependent. For example, the preparation, measurement, and analysis for the WBC differential is different from that for platelets, reticulocytes, lymphocyte subsets, etc. General steps, however, are common to each phase.

Detailed Description Text (362):
In the first phase, automated sample preparation, the analyzer 64 aspirates a volume of the sample, transports the sample to designated cups, and mixes the sample with diluent and/or reagent as required to prepare the sample for measurement. The preparation may only involve diluting the sample, and the diluting means may also be the lysis for removing RBCs. Sometimes, as in the reticulocyte test, the preparation phase involves two steps, a first step predilution with a diluent/sheath reagent, and a second step dilution adding a known volume of fluorescent stain.

Detailed Description Text (367):
The testing protocol for the sample preparation and measurement phases of sample processing are implemented automatically by means of flow sequences, which vary in complexity. In tests involving extended incubation, the flow sequence integrates the incubation and non-incubation testing so that whenever a sample is incubating, the analyzer 64 is allowed to proceed with subsequent tests. When the incubating sample is ready for measurement, processing of further samples is interrupted and the incubated sample undergoes measurement and analysis.

Detailed Description Text (383):
The diluted sample from the RBC cup 134 is also transferred to the optical transducer by valves 236 and 238, pump 232, and syringe 240, 206. The platelets are determined in two dimensional feature space using the PSS (polarized side scatter) and IAS (intermediate angle scatter) optical parameters. The pulses from detectors 384 and 402 are processed, digitized, and stored in list mode files for processing by algorithms. The sample flow rate for measuring platelets is about 2.5 microliters per second, and the counting time through the flowcell is about 6 seconds for normal patients. This counting time is extended automatically for low count samples to improve the count statistics. The count reported from the optical transducer is platelet concentration (PLT).

Detailed Description Text (395):
When cells that meet the triple threshold criteria pass through the illuminated volume, pulses are generated at detectors 382. 384, 400, 402, and 404. The amplitudes of these pulses are filtered, amplified, digitized, and stored in list mode in the corresponding five dimensional feature space of ALL, IAS, FL3, PSS (polarized side scatter), and DSS (depolarized side scatter). The normal counting time through flowcell 170 is about 10 seconds At the flow rate and dilution ratio described, and with a normal patient WBC count of about 7000 cells per microliter of blood volume, the resulting event count rate would be about 5000. In low count samples, this counting time can be automatically extended in order to improve the statistical accuracy of the measurement At the conclusion of the measurement time, the sample stream is piped to waste, and probe 156 is cleaned and dried and prepared to process a subsequent sample.

Detailed Description Text (401):
The measurement process begins as the cell stream intersects the laser illuminated volume at flowcell 170. Data is acquired from optical detectors 382, 384, 400, and 402, via the system electronics and analyzer software and stored in list mode for each Mab/blood reagent mixture. The sample has been diluted so that the cells within the stream pass through the illumination zone of the laser in single file. Each cell is detected by the presence of pulses indicative of four features--ALL(axial light loss), IAS (intermediate angle scatter), FL1 (green fluorescence), and FL2 (orange fluorescence). The amplitude of each pulse is amplified, digitized, and stored in list mode on the appropriate feature space axis.

Detailed Description Text (403):
The normal counting time through flowcell 170 is about 10 seconds. In certain low count samples, this counting time will be automatically extended in order to improve

the counting statistics of the measurement.

Detailed Description Text (405):
The disclosed automated sample preparation features accommodate numerous antibody panels for use in a variety of immunology and phenotyping tests. For lymphocyte subsets, each panel preferably includes five 2-color antibody sets. Preferably, each antibody set incudes one antibody (Mab) marked with FITC (fluorescein isothyocyanate) and the like, and a second Mab marked with PE (Phycoerithrin) and the like. The antibodies are distinguished by cluster designation (CD) numbers. Illustrating by means of example, at least the following lymphocyte subset Mabs may be included in a panel.

Detailed Description Text (407):
In certain other phenotyping Mab tests, the number of Mab pairs, N, might be 1, and hence the required sample volume would be about 50 microliters. Any combination of Mab's may be used. For some tests, the volume of Mab reagent required might be based on an estimate of the WBC patient count obtained from the hematology measurements made on the sample. As for example, in extreme cases of leukocytosis or leukopenia, it may be necessary to adjust the ratio of Mab antibody to patient blood to assure adequate antibody binding or to prevent excess free-antibody background. Because the hematology measurements do not require incubation, they proceed through the flowcell transducer well before the lymphocyte subset sample preparations are completed. The data station can therefore calculate an estimated patient count of the hematology results for that sample to enable the analyzer 64 to adjust as necessary the Mab to blood ratios in order to carry out these tests.

Detailed Description Text (410):
The reagent of the preferred embodiment contains a fluorescent dye with an excitation maximum near the 488 nm argon laser wavelength and a high quantum yield. The preferred reagent stains both DNA and RNA quickly, and in such a way that a single dimension fluorescence histogram avoids the normal WBC confusion. It is so sensitive that the analyzer 64 will detect two fragments of RNA in a cell. The method is linear to up to about 90% reticulocyte count.

Detailed Description Text (416):
Other methods of using the embodiments described herein relate to analyzing cells in a blood sample. These methods are discussed with particular reference to FIGS. 3 (area 114), 4A and 19. There are at least two method of performing immuno-platelet counting. Each of these methods can meet the needs, identified earlier, for improved accuracy and precision in platelet counting by using a flow cytometric method utilizing an optical sensing chamber and light scatter to detect and to count platelets. These methods offer improvements because this type of optical sensing provides for at least two dimensional measurements. This at least two dimensional measurement allows for distinguishment between platelets and other cell fragments that may be present in patient blood. Accordingly, precision of the measurements could be improved.

Detailed Description Text (419):
In this method, cell markers are measured and added to a first volume of sample which is then incubated. When it is desired to perform an immuno-platelet (IP) count, a specimen bar code/work list requisitions the test. At a suitable time, such as the beginning of a week, work shift, etc., an operator operatively connects a container of Mab reagent with the analyzer module. The Mab reagent container may be located in any suitable place, such as adjacent a sample processor block. Upon operative connection of the MAb reagent container with the analyzer module, a data recognition device, such as a bar code reader, a sensor and the like, may verify appropriate connection of the container, i.e. cap removed, access tube installed, etc., and obtain information regarding the MAb reagent and supply that information to the analysis module. The obtained information may be associated with data regarding the patient and/or the patient's sample.

Detailed Description Text (421):
Automated processing of the first patient sample begins. The patient sample may be retained in a container bearing a data carrier, such as a bar code and the like. The patient sample reaches a data recognition device, such as a bar code reader and the

like, associated with the analysis module. The data carrier on the patient sample container is read and the analysis module recognizes a call of an IP count of the patient sample.

Detailed Description Text (594):
22--If a peak at ALL<75 exists, the events with a PSS value greater than the PSS threshold (about 45) are classified as NRBCs and undergo no further analysis.

Detailed Description Text (688):
The validity of the lymphocyte subset measurements described in these Examples is demonstrated by comparing the analysis results using an embodiment of this invention with results of conventional manual flow cytometry assays. The results of such a comparison, between an embodiment of the current invention (termed BB3) and conventional analyses on a FACScan system by Becton Dickinson Immunocytometry Systems, are presented in FIGS. 62A-D.

Other Reference Publication (7):
Cornbleet, MD., J. Spurious Results From Automated Hematology Cell Counters Laboratory Medicine vol. 14, No. 8 Aug. 1983.

CLAIMS:

1. An automated method for distinguishing and differentiating cells in a whole blood sample with an automated instrument system capable of performing both hematology and fluorescent cytometry analysis to which the whole blood sample is provided, upon selection of a series of one or more tests to be performed on the whole blood sample by the automated instrument system, the automated method comprising the steps of:

(g) transporting the first aliquot of diluted whole blood sample through an impedance flow transducer of the automated instrument system;

wherein the instrument system automatically performs all method steps without physically separating cells from the whole blood sample or an aliquot thereof and results of the hematology analysis may be utilized in at least reporting of results of fluorescent cytometry testing.

7. The automated method of claim 1 wherein a reported quantitative platelet result is obtained from multi-angle light scatter and fluorescent detected data.

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L5: Entry 22 of 53 File: USPT

DOCUMENT-IDENTIFIER: US 5874310 A
TITLE: Method for differentiation of nucleated red blood cells

Abstract Text (1):
A method is provided for differentiation of nucleated red blood cells. In addition, the method provides for a concurrent differentiation of leukocytes in a blood cell sample by suitable electronic and optical measurements. The method includes exposing a blood cell sample to a reagent system to lyse mature red blood cells and subsequently analyzing nucleated red blood cells in a flow cell by optical analysis. A concurrent differentiation of nucleated blood cells and leukocytes can be performed using electronic and optical analysis. The electronic and optical analysis includes light scatter and impedance measurements. This method eliminates the use of nuclear stain for identification of nucleated red blood cells. The method of the present invention, for the first time, reports differentiation and enumeration of nucleated red blood cells without using fluorescence.

Brief Summary Text (4):
Normal peripheral blood contains mature red blood cells which are free of nucleus and reticulum. Nucleated red blood cells (NRBCs), or erythroblasts, are immature red blood cells. They normally occur in the bone marrow but not in peripheral blood. However, in certain diseases such as anemia and leukemia, NRBCs also occur in peripheral blood. Therefore, it is of clinical importance to measure NRBC. Traditionally, differentiation and enumeration of NRBC are performed manually. The process involves the smearing of a blood sample on a microscope slide and staining the slide, followed by manual visual analysis of the individual slide. The NRBC concentration is reported as number of NRBCs per 100 white blood cells. Usually, 200 white blood cells and the number of NRBCs present in the same region on a blood smear are counted and the numbers are divided by 2 to express the NRBC concentration as the number of NRBCs/100 white blood cells. This approach is extremely time-consuming as well as being subjective to the interpretation of the individual analyzing the slide.

Brief Summary Text (5):
In recent years, several fluorescence flow cytometry methods have been developed for differentiating NRBCs. These methods utilizes specific nuclear staining technique to distinguish NRBCs from other cell populations because it is not easy to differentiate NRBCs based on their electronic or optical properties.

Brief Summary Text (7):
U.S. Pat. No. 5,559,037 (to Kim et al.) discloses a method for flow cytometric analysis of NRBCs and leukocytes. The method comprises lysis of red blood cells and NRBC cytoplasm from a whole blood sample to expose the NRBC nuclei to a vital nuclear stain and minimizing the permeation of the vital nuclear stain into the leukocytes and analyzing the sample by measuring fluorescence and two angles of light scatter. This method features a triple triggering method which blocks the signals from debris (fluorescent and non-fluorescent) and identifies the signals which fall below the ALL trigger but above the fluorescence trigger (FL3) as NRBCs. ALL is the axial loss of light or the light scatter signals detected at 0.degree. from the incident light. Therefore, pre-gating signals in more than one dimension are required in this method for identification of NRBC population. Since leukocytes are also nucleated cells, staining of these cells needs to be prevented to avoid interference to the fluorescence measurement. The preservation of leukocyte membrane and minimizing the permeation of the nuclear stain into the leukocytes are achieved

by concurrently fixing the leukocytes with an aliphatic aldehyde during lysis of red blood cells. The aldehyde fixatives are known as hazardous chemicals. In addition, the method requires heating of the reagent to 42.degree. C. in order to obtain the NRBC and leukocyte differentiations.

Brief Summary Text (8):
The above described methods are able to differentiate and enumerate NRBCs and leukocytes by fluorescence flow cytometry. However, fluorescence measurement is a complex and expensive detection method.

Brief Summary Text (9):
Current automated hematology analyzers, such as Abbott Cell-Dyn.RTM.3500, COULTER.RTM. STKS.RTM., Technicon H*1.RTM. and TOA Sysmex.TM. NE-8000 are only able to provide NRBC flagging for the possible presence of NRBCs in an analyzed blood sample when the instruments sense an increased amount of signals near red blood cell debris area of a histogram. However, such techniques frequently generate false positive flagging because many other blood abnormalities can cause increased signals at the same area, such as platelet clumps and sickle cells, as well as red cell debris from insufficiently lysed blood samples. In these methods NRBCs are not identified. Instead, only a common NRBC sample distribution pattern in a histogram or a dotplot is recognized by the instrument which can be easily confused with a similar pattern generated by above-mentioned other causes. For the flagged samples, including false positive flags, re-examination of the sample with manual method is required in clinical laboratories. Another problem with the NRBC containing samples is that the white blood cell count (WBC) reported by hematology analyzers is not accurate for these samples since NRBCs could elevate the WBC by being misidentified as white cells. On the other hand, analysis of leukocyte populations from whole blood samples is an integral part of diagnostic procedures regarding a multiplicity of pathologies. The ability to analyze the major subpopulations of leukocytes in an automated manner is essential for a rapid diagnosis of a single blood sample and for the rapid processing of many samples at once.

Brief Summary Text (10):
U.S. Pat. No. 5,155,044 (to Ledis et al.) discloses a method for isolation and analysis of leukocytes from a whole blood sample, which enables differentiation of leukocytes into five subpopulations in a one-step measurement on an automated hematology analyzer. The detection technique involves a concurrent light scatter measurement and impedance measurements in both DC (direct current) and RF (radio frequency). This method is simple and fast, but it does not provide differentiation of NRBCs.

Brief Summary Text (12):
U.S. Pat. No. 5,686,308 (to Li et al.) describes a lysing reagent system and a method for differentiation of leukocytes into five subpopulations in a one-step measurement on an automated hematology analyzer. The lytic reagent comprises a lytic reagent comprising an ethoxylated long chain amine compound and acid to adjust the pH of the lytic reagent to be within the range of 2.0 to 3.6; and a hypertonic, alkaline stabilizing reagent. This patent teaches a reagent and method for differentiation of leukocytes subpopulations, but does not teach differentiation of nucleated red blood cells.

Brief Summary Text (15):
One object of the present invention is to provide a method which permit the differentiation of nucleated red blood cells on an automated hematology analyzer without using fluorescence or nuclear stain. The method comprises exposing a blood cell sample to a reagent system to lyse mature red blood cells; analyzing said sample in a flow cell by light scatter measurement to differentiate nucleated red blood cells; and reporting nucleated red blood cells in said blood cell sample.

Brief Summary Text (16):
Another object of the present invention is to provide a method which permits a concurrent differentiation of nucleated red blood cells and leukocytes. The method comprises exposing a blood cell sample to a reagent system to lyse mature red blood cells without damaging leukocytes; analyzing the sample in a flow cell by DC and light scatter measurements to differentiate nucleated red blood cells and leukocyte

subpopulations; and reporting NRBCs and leukocyte subpopulations in the blood cell sample.

Detailed Description Text (3):
In a first embodiment, the method of the present invention comprises exposing a blood cell sample to a reagent system to lyse mature red blood cells; subsequently analyzing the sample mixture in a flow cell using light scatters to differentiate NRBCs; and reporting NRBCs in the blood cell sample.

Detailed Description Text (18):
In a second embodiment of the present invention, a differential analysis of leukocytes can be performed together with the differentiation of NRBCs. The differential analysis can be performed using the same reagent system and one sample preparation, in a one step measurement using electronic and optical analysis. The electronic and optical analysis includes light scatter and impedance measurements. The DC impedance measurement device used for leukocyte analysis by an automated hematology analyzer is known to those skilled in the art and is generally described in U.S. Pat. No. 5,125,737, to Rodriguez et al., which is hereby incorporated by reference in its entirety. For differential analysis of NRBC and leukocytes a light scatter detector capable of detecting low and medium angle light scatter signals from a particle or a cell passing through the flow cell is used.

Detailed Description Text (21):
The method of the present invention, for the first time, reports the differentiation and enumeration of nucleated red blood cells without using nuclear stain and fluorescence. Elimination of nuclear staining provides advantages in reducing instrument and fluid system maintenance because of dye contamination, reducing system's sensitivity to reagent carry-over and reducing reagent cost due to expensive fluorescent dyes. The method of the present invention is fast, reliable and suitable for automated blood analysis. In addition, the detection technique is less complex and inexpensive compared to fluorescence method.

Detailed Description Text (26):
To 28 .mu.l of an EDTA-anticoagulated clinical whole blood sample 417 .mu.l of a lytic reagent comprising 0.18% formic acid, 2% of ethoxylated long chain amine represented by formula: ##STR2## wherein R is stearyl and m+n is equal to 27, 1.4% of Plurafac A38 as a solubilizer and preservatives were added and mixed in a mixing chamber on an experimental hematology analyzer for about 4 seconds. Then 180 .mu.l of a stabilizing reagent comprising 1.4% NaCl, 3.2% Na.sub.2 SO.sub.4 and 0.66% Na.sub.2 CO.sub.3, and having a pH of 11.0 was added and mixed to retard the lytic reaction.

Detailed Description Text (27):
Ten seconds after the addition of the stabilizing reagent the sample mixture was delivered to a flow cell with a sheath fluid, ISOTON.RTM. III diluent (product of Coulter Corporation, Miami, Fla.), for NRBC and leukocyte differential analysis on an experimental hematology analyzer equipped with DC and light scatter detectors. The light scatter detector detects light scatter signals from a cell passing through the flow cell at several ranges of angles, i.e., from about 1.degree. to about 3.degree. (LS1), from about 4.degree. to about 6.degree. (LS2), from about 24.degree. to about 35.degree. (LS3) and higher angles.

Detailed Description Text (31):
A fresh normal whole blood sample was analyzed using the same reagents and procedure described in Example I. The sample mixture was analyzed simultaneously in the flow cell on the same hematology analyzer used in Example I for leukocyte differentiation and NRBC differentiation. FIG. 2 is an obtained DC vs. LS3 scattergram, which shows four distinct clusters of leukocyte subpopulations, lymphocytes, monocytes, neutrophils and eosinophils.

Detailed Description Text (33):
To 28 .mu.l of an EDTA-anticoagulated clinical whole blood sample 449 .mu.l of a lytic reagent composed of 0.12% formic acid and 0.07% of saponin were added and mixed in a mixing chamber on a hematology analyzer for about 5 seconds. Then 177 .mu.l of the same stabilizing reagent described in Example I were added and mixed to

inhibit the lytic reaction.

Detailed Description Text (34):
About 8 seconds after the addition of the stabilizing reagent, the sample mixture was delivered to a flow cell with a sheath fluid, ISOTON.RTM. III diluent for NRBC and leukocyte differential analysis on the same hematology analyzer described in Example I. FIG. 3 is the resultant LS1 vs. LS2 scattergram (in the same scale as in FIG. 1) which shows the NRBC cluster separated from other cell types.

Detailed Description Text (40):
4 .mu.l of an alligator blood was mixed with 4.15 ml of the lytic reagent of Example I in a test tube manually for about 4 seconds. 1.8 ml of the stabilizing reagent of Example I was added and the sample mixture was mixed manually and introduced to the hematology analyzer described in Example I. FIG. 7 is the obtained LS1 vs. LS2 scattergram. As shown, the NRBCs formed a distinct cluster.

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L5: Entry 24 of 53 File: USPT

DOCUMENT-IDENTIFIER: US 5858667 A
TITLE: Method for the enumeration of micronucleated erythrocyte populations with a single laser flow cytometer

Abstract Text (1):
A single laser flow cytometric method for the enumeration of micronuclei in erythrocyte populations, wherein a sample of peripheral blood or bone marrow is obtained and the cell populations in the sample are fixed. Reticulocytes in the fixed samples are treated simultaneously with RNAse and with a fluorescent labeled antibody having binding specificity for a surface marker for erythroblasts/reticulocytes. The erythrocyte populations are then stained with a nucleic acid stain which stains DNA representing micronuclei, if present. The stained and/or labelled erythrocyte populations are then exposed to a laser beam of appropriate excitation wavelength for both the nucleic acid staining dye and the fluorescent label to produce fluorescent emission. The fluorescent emission and light scatter produced by the erythrocyte populations are detected by the flow cytometer from which is calculated the number of specific erythrocyte populations in said sample.

Brief Summary Text (2):
This invention is directed to medical applications, and the field of toxicology, in which a need exists for a rapid, sensitive and economical method for evaluating micronucleated erythrocytes. More particularly, the present invention relates to a process for analyzing the frequency of micronuclei in erythrocyte populations from peripheral blood or bone marrow by a rapid and sensitive single-laser flow cytometric method.

Brief Summary Text (4):
The in vivo micronucleus test, as performed in mice, has gained widespread use as a short-term system to screen chemicals for clastogenic (chromosome-breaking) activity. The test is based on the observation that mitotic cells with either chromatid breaks or dysfunctional spindle apparatus exhibit disturbances in the anaphase distribution of their chromatin. After telophase, this displaced chromatin can be excluded from the nuclei of the daughter cell and is found in the cytoplasm as a micronucleus. Traditionally, micronuclei were scored in bone marrow preparations. An important advance came with the observation that micronucleated erythrocytes are not cleared from the blood of mice, thus allowing the analysis to be carried out more readily with peripheral blood samples. Erythrocytes are particularly well suited for evaluating micronuclei events since the nucleus of the erythroblast is expelled a few hours after the last mitosis yielding DNA deficient cells. Consequently, micronuclei are particularly apparent in this cell population which is otherwise devoid of DNA. Treatment with clastogens and/or spindle poisons which cause genotoxic damage to stem cells results in the formation of easily detectable micronuclei in these young anucleated reticulocytes. These young anucleated cells are still rich in RNA and certain surface markers (e.g., CD71) and with appropriate staining can be distinguished from the mature normochromatic erythrocytes. From the bone marrow, these reticulocytes enter the bloodstream where they complete their evolution to RNA deficient normochromatic erythrocytes. By scoring micronuclei exclusively in the short-lived reticulocyte population, variation to micronuclei frequency can be attributed to a recent cell cycle, making the system amenable to acute exposure protocols.

Brief Summary Text (6):

The spontaneous background level of micronuclei in blood cells is usually quite low (approximately 2 micronuclei/1000 cells). The rarity of the micronuclei events coupled with the low throughput capacity of microscopic scoring procedures makes the conventional assay labor intensive and time consuming. The scoring operations are subject to human errors arising from the level of experience of each technician. Furthermore, assay sensitivity may be low due to the relatively small number of cells that are processed using the traditional microscopy-based scoring procedure. Manually scoring the slides for micronuclei takes weeks, leading to a considerable level of fatigue. Practitioners of the art realize the need for automated methods to objectively and accurately score larger numbers of micronucleated cells thereby improving assay sensitivity and reliability.

Brief Summary Text (7):
At the present time in this art, the most rapid and accurate way to enumerate micronucleated erythrocytes in the total peripheral blood erythrocyte pool is by a flow cytometric method. One such method is disclosed in U.S. Pat. No. 5,229,265 (to the same Assignee hereof, the disclosure of which is herein incorporated by reference). In a flow cytometric method, cells pass in single file through a laser beam where their fluorescence and light scatter properties are determined. In contrast to manual methods where only 1000-2000 cells per sample are scored, modern flow cytometers are capable of processing cells at rates in excess of 8,000 cells/second. By evaluating more cells, greater scoring accuracy is achieved. A considerable challenge has been to develop reliable automated methods for quantitating micronuclei events in peripheral blood and bone marrow reticulocytes. The advantage of restricting the analysis to these newly formed cells is that this population can highlight genotoxic or cytogenetic action resulting from acute exposures.

Brief Summary Text (8):
Classically, reticulocytes are divided into five populations which are defined by the staining pattern observed in the presence of RNA-precipitating dyes. Stains such as thiazole orange (Lee et al., 1986, Cytometry 7:508-516) and acridine orange (Seligman et al., 1983, Am. J. Hematology 14:57-66) are widely employed. However, in regards to a flow cytometry-based micronucleus assay, these and other RNA dyes are problematic. Since RNA dyes actually bind to DNA as well, overlapping signals tend to limit the resolution of micronucleated reticulocytes from micronucleated normochromatic erythrocytes. A flow cytometric method utilizing a dual dye combination consisting of thiazole orange and Hoechst 33342 has been described (Grawe et al., 1992, Cytometry 13:750-758). Thiazole orange stains the RNA component of the reticulocyte population, and Hoechst dye is used to label micronuclei. The dissimilar wavelengths necessary for the excitation of DNA and RNA dyes necessitates the use of a dual-laser flow cytometer.

Brief Summary Text (9):
Accordingly, there is a need in this art for a rapid, simple and accurate technique to determine the changes in the micronucleated cell populations in the blood and bone marrow cells caused by the action of clastogenic agents. Such a technique would desirably use reticulocyte and micronuclei-specific labels that are excited by a similar wavelength but exhibit significantly different emission spectra, thus enabling the use of a single-laser flow cytometer in a flow cytometric-based micronucleus assay.

Brief Summary Text (11):
An object of this invention is to provide a single-laser flow cytometric method for simultaneously and separately quantitating micronuclei events in the mature normochromatic erythrocyte population and the immature reticulocyte population. In the method of the present invention, fluorescent-labelled antibodies directed against a surface marker for erythroblasts (representing a certain differentiation stage of erythroid cells) are used to label the reticulocyte population, and a nucleic acid staining dye is utilized to resolve the micronuclei events. An important advantage of this procedure is that these reticulocyte and micronuclei-specific labels are excited by a similar wavelength, but exhibit significantly different emission spectra, thus enabling the use of a single-laser flow cytometer in the flow cytometry-based micronucleus assay according to the present invention.

Brief Summary Text (13):
Another object of the present invention is to provide a flow cytometry-based micronucleus assay that supplies repeatable and reliable data with technical ease and modest equipment requirements. The method described herein allows for the flow cytometric analysis, using a single-laser flow cytometer, of the micronucleated normochromatic erythrocyte and micronucleated reticulocyte cell populations in peripheral blood samples or in bone marrow preparations. The invention provides details concerning sample preparation and instrument configuration. The process is able to analyze thousands of erythrocytes in each blood or bone marrow sample in minutes, thereby enhancing the accuracy of the measurements.

Detailed Description Text (3):
By the term "erythrocyte populations" is meant, for the purposes of the specification and claims to refer to populations of mature normochromatic erythrocytes, immature erythrocytes such as erythroblasts and/or reticulocytes, micronucleated normochromatic erythrocytes, micronucleated reticulocytes, and a combination thereof from peripheral blood or bone marrow origins. By the term "a surface marker for erythroblasts/reticulocytes" is meant, for the purposes of the specification and claims to refer to at least one species of a surface molecule present on reticulocytes but absent on mature erythrocytes, thereby enabling reticulocytes to be distinguished from mature erythrocytes by the presence of this marker. Such markers are known in the art to include, but are not limited to, CD71 (a transferrin receptor; Sancho et al., 1994, Biosci. Rep. 14:119-130); a 69 kDa molecule recognized by monoclonal antibody MAE15 (Tonevitsky et al., 1986, Int. J. Cancer 37:263-273); a molecule recognized by monoclonal antibody FA6-152 (Edelman et al., 1986, Blood 67:56-63); a molecule(s) recognized by monoclonal antibodies HAE3 and HAE9 (Ievleva et al., 1986, Eksp Onkol 8:27-28); a molecule referred to as Ag-Eb, antigen of erythroblasts (Ievleva et a., 1976, Int. J. Cancer 17:798-805); and an antigenic determinant recognized by monoclonal antibody 5F1 (Andrews et al., 1983, Blood 62:124-132). One or more of these markers are present on mammalian species other than mice. For example, CD71 is present on human and rat reticulocytes but absent on mature erythrocytes. As illustrated by the above-listed references, it is a standard procedure known to those skilled in the art to make a monoclonal antibody specific for such a marker (see, e.g., Edelman et al., wherein mice were immunized with fetal erythrocytes). Such monoclonal antibodies may be useful in the single-laser flow cytometric method-based micronucleus assay according to the present invention.

Detailed Description Text (7):
The process of this invention is broad in scope and, consequently, the preferred embodiments cover many disciplines which lead to reliable and reproducible flow cytometry-based micronucleus analyses. The description of the preferred embodiment pertains to procedures necessary to analyze erythrocyte populations for the presence of micronuclei. The description will therefore directly parallel the operation that would be carried out in a typical micronucleus assay.

Detailed Description Text (9):
Fixing Blood Cells For Analysis By Flow Cytometry

Detailed Description Text (10):
A suitable and reproducible fixing procedure is necessary to provide cells from erythrocyte populations that are compatible with subsequent staining and analysis by flow cytometry. For the present invention, the fixing procedure must provide cells with the following characteristics: (1) in suspension and free of aggregates; (2) permeability to nucleic acid staining dyes and RNAse; (3) bearing a CD71 antigen or other surface marker for erythroblasts/reticulocytes recognizable by a respective antibody; and (4) low autofluorescence. While there may be several fixing procedures known to those skilled in the art that are suitable for use with the method according to the present invention, a description of a preferred fixing procedure follows.

Detailed Description Text (17):
Flow Cytometer Setup

Detailed Description Text (18):
Optimum flow cytometer settings are extremely important in this process. The fluorescent signals of the stained micronucleated normochromatic erythrocyte and micronucleated reticulocyte populations should be clearly resolved from those of the normochromatic erythrocyte and reticulocyte cell populations. In accordance with the method of the present invention, the analyses described herein were carried out with a single laser flow cytometer (Fac Star.sup.PLUS .TM., Becton Dickenson). The 5 W argon ion laser was tuned to provide 488 nm excitation. Cells were passed through the laser at an average rate of 2,500-8,000 erythrocytes/second. One embodiment of the invention is to use a 20.mu. elliptical beam that is focused at the sample stream. The results and analyses described herein were obtained with said beam. As known to those skilled in the art, different beam shapes are also acceptable.

Detailed Description Text (19):
The flow cytometer is equipped with four photomultiplier tubes (PMTs) which are used to sense forward light scatter, side light scatter, red fluorescence signals, and green fluorescence signals. Proper filters must be placed before the green and red photomultiplier tubes. In accordance with the methods of the present invention, adequate fluorescent signals can be obtained if a 520 nm long pass filter and a 555 nm short pass filter is placed before the green PMT. In accordance with the methods of the present invention, a 580 nm long pass filter in front of the red PMT is desirable. This is a preferred configuration that allows optimal fluorescence detection. However, as known to those skilled in the art, other filter sets may be acceptable. The signals received by the PMTs and the light scatter photodiode are processed by an in-line computer and cell populations of interest are quantitated (see, e.g., FIG. 1) using software conventional to the art. The data may be acquired and processed as either logarithmic or linear signals. In one configuration of the invention, log data was acquired for the forward light scatter, side scatter, as well as red and green fluorescence peak height signals.

Detailed Description Text (20):
Erythrocytes were isolated by gating on forward and side light scatter parameters. The stop mode of each analysis was reached when 500,000 total erythrocytes were interrogated. A window was set up corresponding to cells low in both fluorescence due to the labelled antibody with binding specificity for a surface marker for erythroblasts/reticulocytes (e.g., anti-CD71 antibody) and fluorescence due to the nucleic acid staining dye (e.g., propidium iodide), such as for detecting normochromatic erythrocytes in the erythrocyte population. A second gate was used to define cells low in anti-CD71 fluorescence and high in propidium iodide fluorescence, such as for detecting micronucleated normochromatic erythrocytes in the erythrocyte population. Another region corresponding to cells which were high in anti-CD71 fluorescence and low in propidium iodide fluorescence, such as for detecting reticulocytes in the erythrocyte population. A fourth region corresponding to cells which were high in both anti-CD71 fluorescence and in propidium iodide fluorescence was defined for detecting micronucleated reticulocytes in the erythrocyte population. Having defined these regions, the frequency of reticulocytes, micronucleated reticulocytes, micronucleated normochromatic erythrocytes, and normochromatic erythrocytes were automatically determined by the flow cytometry software.

Detailed Description Text (23):
It is known to practitioners in the art that reticulocytes express certain surface markers which can be used to distinguish reticulocytes from mature erythrocytes. One such marker is the CD71-defined antigen, the transferrin receptor. Fluorescent anti-transferrin receptor antibodies have been used to differentially stain and score reticulocytes via flow cytometry technology (Seligman et al., 1983, Am. J. Hematology 14:57-66; Serke et al., 1992, British J. Haematology 81:432-439). Various protocols are known in the art for labeling antibody with a fluorescent label. For example, a general protocol for labeling the antibody with FITC is as follows. The antibody, in a concentration of from 0.1 to 10 mg/ml, is dissolved in 0.1 to 0.2M bicarbonate buffer (pH 8.3 to 9). The antibody is then incubated with FITC (1 mg/ml) with continuous stirring for 1 hour at 22.degree. C. in a labeling buffer of 0.1M sodium bicarbonate (pH 9.0). The reaction is stopped with hydroxylamine (0.15M final concentration, freshly prepared), incubated for 1 hour, after which the labeled antibody is dialyzed extensively in the dark at 4.degree. C., and then stored at

-75.degree. C. until used. Alternatively, to remove unincorporated fluorescent label from the labelled antibody can be subjected to molecular sieve chromatography.

Detailed Description Text (24):
In further illustrating this embodiment, experiments were performed to optimize the resolution of the fluorescein isothiocyanate conjugated anti-CD71 antibody (purchased commercially) labelled reticulocytes. For this experiment, 20 .mu.l of fixed cells were added to 80 .mu.l of working FITC-anti-CD71 antibody solutions covering a range of concentrations (0-30 .mu.l stock FITC-anti-CD71 antibody/ml bicarbonate buffer). After 30 minutes at 4.degree. C., 1 ml bicarbonate buffer was added and the cells were analyzed via flow cytometry to evaluate effective FITC-anti-CD71 antibody concentrations. The PMT voltage settings of the flow cytometer were adjusted to provide maximum resolution of the reticulocyte population. The results, shown in Table 1, suggest that with these conditions, the concentration of FITC-anti-CD71 antibody in the working solution may be between 5 .mu.l and 30 .mu.l stock FITC-anti-CD71 antibody/ml bicarbonate buffer.

Detailed Description Text (25):
In a preferred method, 20 .mu.l of the fixed cell suspension is added to 80 .mu.l working FITC-anti-CD71 antibody solution (10 .mu.l stock FITC-anti-CD71 antibody/ml bicarbonate buffer) and incubated at 4.degree. C. After 30 minutes, 1 ml of bicarbonate buffer is added and the cells are ready for flow cytometric analysis. By performing the labelling procedure in a low volume, the need to centrifuge and wash cells is eliminated.

Detailed Description Text (27):
Each of the 6 blood samples collected from the 2 animals was fixed and analyzed for reticulocyte content via flow cytometry. These measurements were determined using two methods. The first method employed the nucleic acid staining dye propidium iodide. Similar to new methylene blue or acridine orange, propidium iodide differentially stains the immature erythrocytes based on their RNA content (Wallen et al., 1980, Cytometry 3:155). For this analysis, 20 .mu.l of fixed blood cells were transferred to tubes containing 1.25 .mu.g propidium iodide/ml bicarbonate buffer. The samples were analyzed with a flow cytometer providing 488 nm excitation. For each measurement, 500,000 total erythrocytes were interrogated, and the population of cells expressing a high red fluorescent signal (e.g., above channel 150) were scored as RNA-positive reticulocytes. The second method utilized the immunofluorescent reagent, FITC-anti-CD71 antibody. For this analysis, one preferred method involves adding 20 .mu.l aliquots of fixed blood cells to tubes containing 80 .mu.l working FITC-anti-CD71 antibody solution (10 .mu.l stock FITC-anti-CD71 antibody per ml bicarbonate buffer). The cells were placed at 4.degree. C. for 30 minutes, resuspended with 1 ml cold bicarbonate buffer, and analyzed with 488 nm excitation. As with the propidium iodide analyses, 500,000 erythrocytes were interrogated per blood sample. The population of erythrocytes expressing a high green fluorescent signal (e.g., greater than channel 150) were scored as reticulocytes.

Detailed Description Text (31):
Propidium iodide and FITC have been employed to simultaneously label nucleic acids and cell surface antigens in human B-cell lymphomas (Kruth et al., 1981, Cancer Res. 41:4895-4899; Montecucco et al., 1985, Basic Appl. Histochem. 29:275-2821). In the method according to the present invention the combined use of a nucleic acid staining dye, such as propidium iodide, and FITC-antibody labeling is utilized to differentially stain micronucleated reticulocytes. For this procedure, fixed cells (erythrocyte populations) are washed out of methanol as described and stored at 4.degree. C. In a preferred embodiment of the method according to the present invention, 20 .mu.l aliquots of fixed cells are added to tubes containing 80 .mu.l working FITC-anti-CD71 antibody solution with 1 mg ribonuclease A/ml bicarbonate buffer (RNAse A). This solution prepares the cells for scoring by simultaneously labeling reticulocytes with FITC-anti-CD71 antibody and eliminating RNA content. Other RNAse A concentrations are acceptable, but it is necessary for all the RNA to be degraded prior to analysis by this flow cytometric method. Other buffers, such as phosphate-buffered saline, are known to those skilled in the art. After 30 minutes at 4.degree. C. for fixed erythrocytes from peripheral blood samples (and approximately several hours or more at 4.degree. C. for fixed cells from bone marrow

preparations), 1 ml cold nucleic acid dye staining solution is added (e.g., 1.25 .mu.g propidium iodide/ml bicarbonate buffer). Note that degradation of reticulocytes' RNA has to be achieved so that fluorescence emitted by the nucleic acid staining dye represents a DNA (micronuclei) specific signal. Following the addition of the nucleic acid staining dye solution, cells are kept at 4.degree. C. until analysis.

Detailed Description Text (34):
When quantitatively analyzing rare events such as the micronucleated reticulocyte population, it is critical that the flow cytometer's sample tubing is clean and free of debris which may interfere with highly accurate scoring. In that regard, it may be desirable to run a particle-free solution consisting of 1% bleach with 50 mM NaOH in dH.sub.2 O through the sample line for approximately one minute before each sample.

Detailed Description Text (38):
To closely evaluate the effectiveness and reliability of this automated scoring method, an experiment was designed to track the incidence of micronuclei in the peripheral blood of mice after an acute (single) exposure to clastogen. Immediately after obtaining initial (time 0 hour) blood samples from 5 male mice, each animal was injected with 100 mg MMS/kg body weight. Clastogen was delivered in a volume of 25 .mu.l/kg body weight. Subsequent blood samples were collected at 24, 40, 48 and 72 hours. Blood samples were fixed and stored at -70.degree. C. until completion of the experiment.

Detailed Description Text (41):
Aside from comparing micronuclei frequencies over time, it is informative to study the frequency of micronuclei events in the set of 0 hour samples. As indicated by Table 2, the mean frequency of micronucleated normochromatic erythrocytes and micronucleated reticulocytes are very similar at time 0 hour (0.25% versus 0.27%, respectively). We would expect these values to be approximately equal, since mice do not effectively clear micronuclei from circulation. Note that before optimal staining and flow cytometric operating procedures were realized, these values often diverged significantly. Thus, for the experimental model, a correspondence between these numbers is one useful tool which provides evidence that micronucleated normochromatic erythrocytes and micronucleated reticulocytes populations are being scored reliably and with precision.

Detailed Description Text (42):
To evaluate the consistency of measurements obtained by the method according to the present invention, an experiment was performed with blood samples obtained from MMS mouse no. 1. For this evaluation, each of the animal's 5 blood samples were prepared for flow cytometric analysis using the method according to the present invention, and scored one day and again seven days after the initial measurements reported in Table 2. These data are graphically presented in FIG. 4, wherein measurements from day 0 (--.largecircle.--) are compared to those from day 1 (--.quadrature.--) and from day 7 (--.DELTA.--). The high reproducibility of the resultant measurements suggest that fixed samples can be stored at 4.degree. C. for at least one week without appreciable loss in scoring reliability, and support the consistency of the flow cytometric scoring when using the method according to the present invention.

Detailed Description Text (43):
The data reported for the MMS experiment suggest that the analysis windows used to define the micronucleated normochromatic erythrocytes and micronucleated reticulocyte populations are highly appropriate. Over the 72 hour experimental time-frame, the incidence of micronuclei in the micronucleated normochromatic erythrocyte population rose slightly, and the frequency of micronucleated reticulocytes was observed to rise and fall quickly. Given the persistence of these populations in the peripheral blood pool of mice, these profiles are expected. Furthermore, the high reproducibility found when blood samples from an MMS-treated mouse were re-analyzed over the course of a week suggests that the analysis windows can be used to compare flow cytometric data obtained on different days.

Detailed Description Text (44):
From the foregoing description, one skilled in the art will be capable of analyzing

peripheral blood or bone marrow cell preparations for the presence of micronucleated normochromatic erythrocytes and micronucleated reticulocytes by flow cytometry. Obvious modifications and variations, such as substitution of equivalents or adaptation for various applications, will be apparent to one skilled in the art from the foregoing description, and such are considered within the scope of the claimed invention.

Detailed Description Paragraph Table (2):
TABLE 2 ______________________________________ Flow cytometric analysis of MMS-induced micronuclei. No. No. Time Mouse No. MN- Freq. (%) No. MN- Freq. (%) (hrs) No. NCE NCE MN-NCE RET RET MN-RET ______________________________________ 0 1 490631 1339 0.27 8471 26 0.31 2 490648 1240 0.25 8541 23 0.72 3 489526 1111 0.23 9825 24 0.25 4 491259 1150 0.23 7883 21 0.27 5 493156 1390 0.28 6030 16 0.27 Average 0.25 0.27 24 1 490949 1244 0.25 8531 46 0.54 2 490112 1287 0.26 9390 88 0.93 3 488089 1145 0.23 11532 80 0.69 4 487462 1254 0.26 12234 162 1.31 5 490011 1391 0.28 9391 55 0.58 Average 0.26 0.81 40 1 490511 1405 0.29 8373 386 4.41 2 485301 1285 0.26 13570 592 4.18 3 486302 1178 0.24 12679 652 4.89 4 484237 1445 0.30 14533 581 3.81 5 492350 1458 0.30 6785 262 3.72 Average 0.28 4.20 48 1 487164 1515 0.31 11794 355 2.92 2 481651 1538 0.32 17187 366 2.09 3 480648 1298 0.27 18484 396 2.10 4 476364 1517 0.32 22687 431 1.86 5 490250 1339 0.27 8990 211 2.29 Average 0.30 2.25 72 1 473052 1593 0.34 25838 112 0.43 2 462539 1573 0.34 36327 147 0.40 3 450096 1607 0.36 48913 224 0.46 4 461882 1628 0.35 36940 150 0.40 5 475141 1575 0.33 23744 82 0.34 Average 0.34 0.41 ______________________________________ Abbreviations: NCE = normochromatic erythrocytes; MN - NCE = micronucleated normochromatic erythrocytes; RET = reticulocytes; MN - RET = micronucleated reticulocytes

Other Reference Publication (1):
Tometsko et al., "Analysis of micronucleated cells by flow cytometry. 4. Kinetic analysis of cytogenetic damage in blood," Mutation Research, vol. 334, No. 1, pp. 9-18, Jan. 1995.

Other Reference Publication (2):
Dertinger et al., "Simple and reliable enumeration of micronucleated reticulocytes with a single-laser flow cytometer," Mutation Research, vol. 371, Nos. 3,4, pp. 283-292, Dec. 20, 1996.

Other Reference Publication (3):
Begg et al., "Cell Kinetic Analysis of Mixed Populations Using Three-Color Fluorescence Flow Cytometry," Cytometry, vol. 12, No. 5, pp. 445-454, 1991 .

CLAIMS:

1. A single laser flow cytometric method for the enumeration of micronuclei events in erythrocyte populations, the method comprising the steps of:

(d) staining in the erythrocyte populations DNA representing micronuclei with a nucleic acid staining dye in a concentration range detectable by flow cytometry;

2. The flow cytometric method according to claim 1, wherein the sample is from peripheral blood.

3. The flow cytometric method according to claim 1, wherein the sample is from bone marrow.

4. The flow cytometric method according to claim 1, wherein the primary alcohol is ethanol.

5. The flow cytometric method according to claim 4, wherein the primary alcohol is methanol.

6. The flow cytometric method according to claim 1, wherein the fluorescent labeled antibody having binding specificity for a surface marker for erythroblasts/reticulocytes is FITC-anti-CD71 antibody.

7. The flow cytometric method according to claim 1, wherein the nucleic acid

staining dye is selected from the group consisting of propidium iodide, ethidium bromide, mithramycin, acridine orange, pyronine Y, and benzathiazolium-4-quinolinium dimer TOTO-1.

8. The flow cytometric method according to claim 7, wherein the nucleic acid staining dye consists of propidium iodide.

9. The flow cytometric method according to claim 1, further comprising administering a clastogenic agent to the individual prior to obtaining a sample of the erythrocyte populations from said individual.

10. The flow cytometric method according to claim 9, wherein the sample is from peripheral blood.

11. The flow cytometric method according to claim 9, wherein the sample is from bone marrow.

12. The flow cytometric method according to claim 9, wherein the primary alcohol is ethanol.

13. The flow cytometric method according to claim 12, wherein the primary alcohol is methanol.

14. The flow cytometric method according to claim 9, wherein the fluorescent labeled antibody having binding specificity for a surface marker for erythroblasts/reticulocytes is FITC-anti-CD71 antibody.

15. The flow cytometric method according to claim 9, wherein the nucleic acid staining dye is selected from the group consisting of propidium iodide, ethidium bromide, mithramycin, acridine orange, pyronine Y, and benzathiazolium-4-quinolinium dimer TOTO-1.

16. The flow cytometric method according to claim 15, wherein the nucleic acid staining dye consists of propidium iodide.

17. The flow cytometric method according to claim 9, further comprising administering a suspected anticlastogen to the individual within relatively the same time period of administration of the clastogenic agent in measuring any protective effect induced by the suspected anticlastogen.

18. The flow cytometric method according to claim 17, wherein the sample is from peripheral blood.

19. The flow cytometric method according to claim 17, wherein the sample is from bone marrow.

20. The flow cytometric method according to claim 17, wherein the primary alcohol is ethanol.

21. The flow cytometric method according to claim 20, wherein the primary alcohol is methanol.

22. The flow cytometric method according to claim 17, wherein the fluorescent labeled antibody having binding specificity for a surface marker for erythroblasts/reticulocytes is FITC-anti-CD71 antibody.

23. The flow cytometric method according to claim 17, wherein the nucleic acid staining dye is selected from the group consisting of propidium iodide, ethidium bromide, mithramycin, acridine orange, pyronine Y, and benzathiazolium-4-quinolinium dimer TOTO-1.

24. The flow cytometric method according to claim 23, wherein the nucleic acid staining dye consists of propidium iodide.

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DOCUMENT-IDENTIFIER: US 5656499 A
TITLE: Method for performing automated hematology and cytometry analysis

Abstract Text (1):
A device for analyzing a whole blood sample is provided. The device comprises a conventionl hematology analyzer integrated with a fluorescence cyometry analyzer. A controller is provided for controlling the analyzers, obtaining and utilizing data from both and reporting a quantitative result. Methods are also provided for analyzing a whole blood sample. One such method comprises the steps of performing on a single instrument an analysis of impedance associated with the blood sample, an analysis of light scatter associated with the blood sample, and an analysis of fluorescence associated with the blood sample. Data is collected and utilized. A result is reported.

Parent Case Text (2):
This is a continuation-in-part application of Ser. No. 08/283,379 filed Aug. 1, 1994 entitled METHOD AND APPARATUS FOR PERFORMING AUTOMATED ANALYSIS, now abandoned. The parent application is assigned to the assignee of this application. The disclosure of the parent application is incorporated herein in its entirety by this reference.

Brief Summary Text (2):
This invention relates in general to particle analysis. More particularly, it relates to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and flow cytometric techniques. This invention also relates to a multipurpose reagent system and a method for rapid analysis of a whole blood sample.

Brief Summary Text (6):
Peripheral blood also contains red cells of earlier maturation levels which are important diagnostic indicators. Two of these are reticulocytes and nucleated red blood cells.

Brief Summary Text (7):
At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an erythroblast. As the erythroblast matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to as nucleated red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in peripheral blood often contribute to inaccurate enumeration of the white cell count, due in part to the presence f a nucleus which makes them difficult to distinguish from small white cells.

Brief Summary Text (8):
Reticulocytes are red cells at the maturation level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs as a result of an uncompensated increase in the rate of removal of erythrocytes from blood, or a decrease in the rate at which they are formed and released into blood. An increased reticulocyte patient count in an anemic patient indicates rapid erythroid turnover which suggests acute blood loss or hemolysis.

Brief Summary Text (16):
In the current state of the art of cell analysis, there are two technologies used

for counting and classifying cells. These are generally known as "flow cytometry" and "image cytometry." The flow cytometry technology, which essentially consists of passing cells one a time through a sensing zone of a flow cell, is preferred in clinical applications where patient test load is an important metric. This is mainly because it has at least an order of magnitude advantage in the number of cells that can be analyzed per second.

Brief Summary Text (17):
Instrumentation incorporating flow cytometry can be further subdivided into two methods which can be generally classified as "conventional hematology" and "fluorescence cytometry."

Brief Summary Text (18):
A primary distinction between the two methods is that conventional hematology generally distinguish cells by means of size and shape alone using primarily impedance and light scatter technologies, whereas fluorescence cytometry uses cell nucleic acid content and/or surface antigens in addition to size and shape in distinguishing cells. Therefore the fluorescence method may be used to subdivide the cell types into finer classifications.

Brief Summary Text (19):
A second distinction between the two methods is that conventional hematology gives results in absolute terms, whereas fluorescence cytometry results are in relative terms. Hematology analyzers deliver precise volumes and dilutions, and are thus able to measure absolute cell concentrations, or absolute counts of cell types per microliter of human blood. The fluorescence cytometry method gives only relative concentrations, or percentages of the various cell types.

Brief Summary Text (20):
A third distinction is that the hematology method is generally automated, whereas the fluorescence cytometric method as generally practiced today, is at best semi-automated, both in sample preparation, and in sample analysis. The fluorescence cytometry method is therefore significantly more labor intensive than the hematology method.

Brief Summary Text (22):
An example of an instrument for performing automated hematology measurements is the Cell-Dyn.RTM. 3000 instrument, which has been sold for several years by Sequoia-Turner, a predecessor in interest of Abbott Laboratories. The Cell-Dyn.RTM. 3000 instrument uses "impedance" measurements to count and size RBCs and PLTs, "absorption" measurements to determine the concentration of hemoglobin in RBCs (MCH), and "optical scatter" measurements to count and classify WBCs and the five part differential.

Brief Summary Text (23):
The Cell-Dyn.RTM. 3000 instrument automatically prepares blood samples, measures cell parameters and generates test results. The complete automation of sample preparation is such that no substantive operator intervention is required once the patient sample of whole blood has been presented to the analyzer. As mentioned previously, in order to assure accurate "patient counts" for the various cell classes, the Cell-Dyn.RTM. 3000 instrument provides precise sample volumes, reagent volumes and dilution volumes. Patient counts are generally defined as the number of "events" per microliter of blood. The events may be RBCs, PLTs, WBCs, and classes or subclasses thereof.

Brief Summary Text (25):
In contrast, the fluorescence flow cytometer incorporates the principles of fluorescence cell analysis with light scatter. In general this requires that the cell be stained with an appropriate color dye, or that a fluorochrome label be attached to an antigen or antibody on the cell's surface thus indicating the occurrence of a specific antigen-antibody reaction.

Brief Summary Text (26):
In fluorescence flow cytometry, a suspension of previously stained or fluorescently labelled particles, typically cells in a blood or other biological fluid sample, is

transported through a flowcell where the individual particles in the sample are illuminated with one or more focused light beams. One or more detectors detect the interaction between the light beam(s) and the labeled particles flowing through the flowcell. Commonly, some of the detectors are designed to measure fluorescent emissions, while other detectors measure scatter intensity or pulse duration. Thus, each particle that passes through the flowcell can be mapped into a feature space whose axes are the emission colors, light intensities, or other properties, i.e. scatter, measured by the detectors. Preferably, the different particles in the sample can be mapped into distinct and non-overlapping regions of the feature space, allowing each particle to be analyzed based on its mapping in the feature space. In this respect, flow cytometry differs from the conventional hematology instruments in that some of the feature space axis includes fluorescence emissions.

Brief Summary Text (27):
As noted above, lymphocyte subclasses are health determinants. Thus, it is desirable that these and other parameters be measured accurately. Although known hematology and fluorescent flow cytometry instruments have made significant advances in the ability to characterize blood cells, a problem still faced in this area is the difficulty in obtaining accurate patient count values for certain classes of cells.

Brief Summary Text (28):
An example of this problem is the CD4 cell patient count. Current analysis methods calculate the CD4 cell patient count from cell parameters measured on a hematology instrument and a separate fluorescence flow cytometry instrument. This calculation can provide up to 100% variability in absolute CD4 patient counts done on a single individual one week apart. See, e.g.: Update, Testing In The Blood Bank, Volume 5, No. 2, pages 1 to 6, published 1991 by Ortho Diagnostics Systems, Inc.

Brief Summary Text (30):
The Lancet, Volume 340, Aug. 22, 1992, page 485 describes variation in CD4 count results when different analyzers are used. The variation appears to stem from different lymphocyte count results.

Brief Summary Text (33):
Laboratory Medicine,August 1983, Volume 14, No. 8, pages 509 to 514 discusses numerous spurious results and their causes in automated hematology analyzers.

Brief Summary Text (35):
One reason for variability in CD4 patient counts is manual sample preparation that cannot be controlled precisely and depends on operator proficiency. For example, a conventional procedure for determining a CD4 patient count starts with drawing two tubes of blood from a patient. One tube is analyzed on a hematology instrument which generates several measured and/or calculated parameters for the blood sample, including a total lymphocyte patient count, a lymphocyte percentage and a total WBC patient count. The second tube of blood is analyzed on a fluorescence flow cytometry instrument. The sample preparation steps for the flow cytometry tests are labor intensive and operator dependent. These steps do not readily lend themselves to automation and precision.

Brief Summary Text (36):
To prepare the sample for the flow cytometry instrument, the operator manually pipettes a volume of blood from the sample tube into an analysis tube. A volume of the desired fluorochrome labeled monoclonal antibody is added. The sample/antibody mixture is then incubated for a predetermined time at a predetermined temperature to allow antibody/antigen bindings to take place. After incubation, the operator adds a volume of RBC lyse to destroy the RBCs in the sample. Timing is important during the lysing stage. If the operator does not allow the lyse reaction to continue long enough, RBCs may remain in the sample and distort the measurements. If the operator allows the lyse reaction to continue for too long, the lyse may attack the WBCs.

Brief Summary Text (37):
After determining that the lyse reaction is complete, the operator centrifuges and washes the sample to remove any debris left over from lysed RBCs. The centrifuge/wash step may be performed several times until the operator is satisfied that the sample is sufficiently clean. Debris, red cell "stroma" can interfere with

the detection processes of the typical flow cytometer. The sample now contains WBCs with antibodies bound to cells bearing the complementary surface antigens. The operator re-suspends the sample in a volume of fixative, and then passes the sample through the fluorescence flow cytometry instrument.

Brief Summary Text (38):
The fluorescence flow cytometry instrument generates only percentage values for lymphocyte subsets. This is at least partially due to the fact that the numerous manual dilutions and volume reductions performed during the sample preparation steps do not allow the isolation of a precise measurement volume. Thus, the fluorescence flow cytometry instrument identifies the CD4 positive helper-T cells as the percentage of lymphocytes which are both positive for CD3 (T cell marker), and positive for CD4 (helper-T marker).

Brief Summary Text (40):
The lymph count and the WBC patient count are taken from the hematology instrument, while the "% helper-T cells in lymph" value is taken from the fluorescence instrument after a correction factor is applied based on the flow cytometer mapping of scatter and fluorescence.

Brief Summary Text (43):
Previous attempts to automate sample preparation in fluorescence cytometry testing have only been partially successful. Such systems still require the operator to perform sample preparation steps such as separating lymphocytes from other peripheral blood cells by density gradient centrifugation, and/or lysing red cells, removing red cell ghosts and cell debris by centrifugation, or preserving the morphology of the remaining white cells by suspending the white cells in an isotonic saline solution containing appropriate fixatives. These operations generally require the operator to manually alter the volume of the sample, thus compromising sample volume precision which can be achieved with automated mechanical volume dispensers.

Brief Summary Text (45):
In leukocyte analyses, it is desirable that all of the RBCS be lysed. Because RBCs outnumber WBCs by about 700 to 1, a small number of unlysed red cells may significantly distort white cell patient counts. Some reagents used to lyse red cells require too lengthy an incubation period to be practical in an automated clinical analyzer. For example, the Tris buffered ammonium chloride solution recommended by K. A. Murihead in Clinical Cytometry, Ann.N.Y. Acd. Sci., vol. 468, pp. 113-127 (1986) takes about 5 to 10 minutes to lyse red cells, which may be impractical for automation.

Brief Summary Text (46):
Furthermore, incomplete hemolysis with certain lytic reagents may result in red cell stroma that retain sufficient hemoglobin or particulate matter to generate high background patient counts in automated clinical electro-optical systems. When this occurs, it is usually necessary to remove the WBCs to be analyzed from the red cell stroma by centrifugation, a procedure that is a limiting factor when adapting a reagent system for automation.

Brief Summary Text (48):
The earliest stage of RBC, the nucleated red cell, NRBC, when found in the peripheral blood on conventional hematology analyzers can be confused for a small lymphocyte, since the lysis will not destroy the nucleus of the NRBC. Because of the ratio of RBCs to WBCs, even a relatively small percentage of NRBCs can lead to substantial error in the WBC and lymphocyte count. This may be troublesome in neonate or pediatric samples, in which the presence of NRBCs in peripheral blood is a normal condition. For this reason, the laboratory may do manual slide inspections on some of these samples. Conventional hematology analyzers are only able to flag these samples by noting the spreading out of the usual lymphocyte scatter cluster. The manual inspection results in a count of the number of NRBCs per 100 nucleated cells. This percentage is then used to correct the analyzer WBC count as follows:

Brief Summary Text (49):
Clearly the need exists for an accurate automated count of NRBCs.

Brief Summary Text (53):
A device for analyzing a whole blood sample is provided. The device, an automated instrument system for distinguishing and differentiating cells in a sample, comprises a conventional hematology analyzer fully integrated with a fluorescence cytometry analyzer. Both analyzers are controlled by a controller which utilizes the results obtained from each analyzer to report test results in absolute, or quantitative terms. Methods are also provided for analyzing a whole blood sample. One such method comprises the steps of automatedly performing both conventional hematology and fluorescence cytometry analysis on a sample. Data is collected and utilized and a quantitative result is reported when appropriate.

Brief Summary Text (54):
A further embodiment provides an automated instrument system comprising a sample handler for receiving a sample container and aspirating and dispensing the sample, a sample analyzer that performs both multi-angle light scatter and fluorescence signal detection, and a controller fully integrating the analyzer to enable the instrument system to report quantitative results of both conventional hematological and fluorescence cytometric results. This instrument system is further able to perform these functions, sequentially if necessary, without any intervention from the instrument operator once the operator has selected an array of tests to be performed by the instrument from a menu presented to the operator, and without separating cells from the sample during any phase of the analyses.

Brief Summary Paragraph Equation (3):
Corrected WBC count=Analyzer count(1-manual NRBC percentage/100)

Drawing Description Text (18):
FIGS. 13A-F comprise a timing diagram illustrating one embodiment of an integrated, automated, hematology/immunology sample processing method of the cell analysis system shown in FIG. 1;

Drawing Description Text (30):
FIG. 25 is a block diagram illustrating one embodiment of the analyzer module of the cell analysis system shown in FIG. 1;

Drawing Description Text (32):
FIG. 27 is a block diagram illustrating further details of the analyzer module shown in FIG 25;

Detailed Description Text (2):
Embodiments of the present invention comprise an analytical instrument system and a method for analyzing fluid samples. Generally, one such automated instrument system includes a conventional hematology analyzer fully integrated with a controller and a fluorescent cytometer. The instrument system is able to distinguish and classify cells, whereby the data collected by the hematology analyzer is automatedly utilized by the fluorescent cytometer to process samples, analyze sample and classify cells within the sample and report quantitative as well as qualitative results.

Detailed Description Text (3):
The automated instrument system herein disclosed combines or integrates conventional hematology with fluorescent cytometry on a single analyzer platform. Heretofore, this approach has not been possible. Both methods benefit by this unique combination. Fluorescence information is improved by total automation and absolute concentrations. The hematology information is enhanced by adding fluorescence cytometry to the technology of colorimetry, impedance, and multi-angle light scatter, thereby enabling superior hematology and total automation of tests which currently are done either manually, or on separate and distinct analyzers.

Detailed Description Text (4):
For the sake of this disclosure, automation is distinguished in that an operator does not need to intervene in the sample preparation process or analysis of the sample, once the sample, i.e., whole blood, urine, saliva etc., is presented to the instrument. Additionally, all sample handling, processing and analyzing steps and functions are carried out automatedly by the instrument based upon the tests selected by the operator. All data and other information pertaining to each initial

test sample is monitored, collected, and processed by the instrument controller.

Detailed Description Text (5):
The embodiments of the invention generally comprise an automated hematology analyzer and a flow cytometry analyzer integrated with a controller which monitors and controls the analyzers, collects data from the analyzers and reports a result. Illustrating by example, integration of the analyzers with a controller allows an operator to input data about a whole blood sample into the controller. The operator selects a series of tests to be performed on the sample, generally whole blood, with the aid of the controller. The operator presents the whole blood sample to the integrated analyzers at a centralized sample handling, or processing area. The controller activates the analyzers, allowing the analyzers to automatedly perform analyses on the whole blood sample under the direction of the controller. The controller utilizes data obtained from the analyzers to formulate a result. The controller reports the result to the operator. It is to be noted that no operator action is needed after the whole blood sample is presented to the integrated analyzers. Because the whole blood sample preparation is entirely automated, in a preferred embodiment, conventional hematology tests are done first with the incubated sample tests to follow. Because the analyzers are integrated with the controller, the controller obtains data from both the hematology analyzer and the flow cytometry analyzer. Thus, the controller is able to report a combined patient blood analysis to the operator. In addition absolute concentrations are reportable because of the precision and repeatability of automated dilution, cell preparation and analysis. Human error has all be been eliminated because the instrument system is the only thing to touch the sample once the operator has programmed the instrument and placed the sample on-board.

Detailed Description Text (8):
FIG. 1 is a block diagram of a cell analysis system 60. The system 60 includes an analyzer module 64, a data station module 68, and a pneumatic unit 72. The analyzer module 64 is operatively connected to the data station module 68 by a serial data link 76 implementing a HDLC (high level data link) protocol. The pneumatic unit 72 is operatively connected to the analyzer module 64 by a serial data link 84 and a network of tubing 80.

Detailed Description Text (9):
The analyzer module 64 aspirates samples, diluent and reagents, dilutes samples, measures and collects data, transmits measured data to the data station module 68, manages reagents, and disposes of waste. An exemplary analyzer module 64 includes its own power supply, impedance transducer, HGB transducer, optical flowcell/transducer (light scattering and fluorescence), optical detectors, electronics, reagent reservoirs, fluidics system, integrated and fully automated sample processor for both hematology and fluorescent cytometry tests, and any necessary incubation and/or cooling systems. An exemplary analyzer module includes a Motorola 68302-type microcomputer that controls mechanical components of the analyzer 64 and executes the analyzer's flow sequences.

Detailed Description Text (10):
The pneumatic unit 72 houses pneumatic sources for moving fluids through the analyzer module 64. The pneumatic unit 72 receives instructions from the analyzer module 64 via that serial data link 84.

Detailed Description Text (11):
The data station module 68 provides general controls to the analyzer module 64, converts measured data into meaningful test results, stores measured data and test results, prints reports, and provides bi-directional communication with an off-line host computer (not shown). An exemplary data station module 68 includes an 80386 or 80486-type microcomputer, color display, 31/2 inch disk drive, at least 540 megabyte hard disk, PC-style keyboard, a pointing device, and LAN connections. The data station 68 includes memory, such as a RAM, a ROM, an EPROM, a SRAM and the like, having sufficient software algorithms to manipulate measured data, calculate parameters, and display results in a variety of formats, including histograms, scattergrams, and other multidimensional plots.

Detailed Description Text (17):

The multipurpose reagent system is utilized in the automated determination of differential white cell patient counts, nucleated red blood cells, and lymphocyte immunophenotyping. A method for the rapid analysis of nucleated peripheral whole blood cells includes the following steps: mixing the described multipurpose reagent system with an anticoagulated whole blood sample (whereby the blood is diluted 10 to 100 fold), mixing the diluent-blood mixture at temperatures from about 25.degree. C. to 46.degree. C. for at least about 10 seconds, and analyzing the nucleated peripheral blood cells with the automated cell analysis system of the present invention.

Detailed Description Text (26):
A further embodiment of the multipurpose reagent system allows for the quantitative analysis of lymphocyte subpopulations. Lymphocyte subclassification is achieved by mixing fluorochrome-conjugated monoclonal antibodies (directed to specific lymphocyte surface antigens) with whole blood samples before adding the multipurpose reagent system, or blood diluent. The concentration of labeled antibody fractions added to a blood sample depends upon the individual antibody preparation, but is commonly about one-half to one-tenth of the volume of the blood for commercial antibody preparations. After the reagent system is added and the red cells are lysed, the lymphocyte-antibody reaction products can be analyzed on an automated flow cytometric system. There is no need to "separate" the lymphocytes from the lysed cells by centrifugation and washing as is common in the art.

Detailed Description Text (29):
The cell analysis system 60 utilizes an automated method for simultaneous analysis of WBC/Diff and NRBC in a whole blood sample using a unique triple triggering method with lyse reagent, such as the rapid lyse reagent system described above. This method, claimed in U.S. patent application Ser. No. 08/356,932, entitled "Method For Rapid And Simultaneous Analysis Of Nucleated Red Blood Cells", and filed on Dec. 15, 1994 enables the accurate NRBC counts and WBC/Diff data, simultaneously from a whole blood sample containing NRBC. The entire contents of U.S. Ser. No. 08/356,932 is hereby incorporated by reference.

Detailed Description Text (32):
The triple trigger method is unique in that the simultaneous analysis of WBC/Diff/NRBC can be carried out automatically, accurately, and rapidly without interference from other cellular debris such as RNA from lysed reticulocytes, Howell Jolly Bodies, reticulated platelets, giant platelets, DNA from WBC and Megakaryocytic fragments, parasites, and RBC fragments.

Detailed Description Text (49):
No matter what the formulation of the lyse utilized with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital nuclear stain which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the herein referenced analyzer, the lysis chemistry will be configured such that the refractive index matches that of a sheath solution to substantially less than 0.1.

Detailed Description Text (53):
When cells, thus triggered, pass through the aforementioned illuminated volume, pulses are generated at detectors 380, 400, 401 and 404. The amplitudes of these pulses are then filtered, amplified, digitized, and stored in list mode in the corresponding five dimensional feature space of ALL, IAS, FL3, PSS (polarized side scatter), and DSS (depolarized side scatter). The normal counting time through flowcell 170 is 10 seconds. At the flow rate and dilution ratio described above, with a normal patient WBC count of 7000 cells per microliter of blood volume, the resulting event count rate would be 5000. In low count samples, this counting time can be automatically extended in order to improve the statistics of the measurement. At the conclusion of the measurement time, the sample stream is piped to waste, and probe is cleaned and dried and prepared to process a subsequent sample.

Detailed Description Text (83):
The method allows the enumeration of reticulocytes from a whole blood sample while simultaneously differentiating a separate aliquot of the sample to obtain a complete blood cell ("CBC") analysis. This method comprises, directing one or more aliquots

of the sample to various positions within an automated analyzer for analysis and differentiation, while a reticulocyte aliquot of the sample is combined with a staining reagent.

Detailed Description Text (84):
The combined reagent/reticulocyte aliquot is then directed to an optical flow cell 170 of the automated analyzer 60. Thereafter the reagent/reticulocyte aliquot is passed through an illuminated sensing zone 300 essentially one cell at a time to cause fluorescence and scattered light events. These events are detected and the number of reticulocytes present in said sample are determined therefrom.

Detailed Description Text (100):
In order to analyze a whole blood sample for the percentage as well as the absolute counts of reticulocytes on the multi-parameter hematology analyzer described above, about 18.75 .mu.l of a whole blood sample is deposited by means of a sample aspiration probe into the RBC cup 134 which contains about 7856 .mu.l of a diluent/sheath solution (an isotonic saline) and the fluids are mixed. The diluted sample is then transported to a sheathed impedance aperture 174 to electronically determine the absolute RBC counts of the sample. In the mean time, about 200 .mu.l of the diluted sample is transferred into Retic cup 136 which contains 600 .mu.l of the disclosed reagent, where it is mixed. The prepared (mixed) sample is then transported to the sheathed optical flow cell 170 for detection. The measurement process begins as the cell stream passes through the flow cell essentially one cell at a time, in a laminar flowing sample stream surrounded by a diluent-sheath solution, disclosed hereinafter.

Detailed Description Text (125):
Another embodiment provides a method for enumeration of reticulocytes in a whole blood sample using flow cytometry wherein an aqueous staining solution of a 2,2'-dye which is capable of staining RNA-containing material is placed in contact with an RNA-containing material for a period of time adequate to enable the staining solution dye equilibrate with the RNA-containing material. The stained sample is then directed through the optical sensing zone of a flow cytometry instrument and illuminated once within the optical sensing zone with an incident light beam. The fluorescence of the reticulocytes in sample solution are then measured as they pass through the optical sensing zone.

Detailed Description Text (129):
The 2,2'-dyes can be used in any conventional assay technique which requires the staining of reticulocytes with a fluorescent marker. In particular, these dyes can be used in any assay for which thiazole orange is currently recommended, such as reticulocyte detection and enumeration in an argon ion laser flow cytometer.

Detailed Description Text (130):
When these class of dyes are utilized to detect and differentiate reticulocytes an incubation site and associated temperature controls and sample handlers must be provided for within the instrument and operatively connected to the analyzer to maintain the automation of the inventive instrument system disclosed herein.

Detailed Description Text (133):
A cyanide-free reagent must be able to quickly lyse the erythrocytes and rapidly complex with the hemoglobin so that a detectable chromogenic structure is formed for detection and measurement. The disclosed reagent is stable for many weeks and is particularly advantageous because the resulting chromogen appears to be free of interference from other blood components and can be measured at wavelengths in the spectral range of automated hematology instruments already in the field. For comparison purposes, the cyan met hemoglobin method typically measures absorbance at 540 nm. A reddish brown chromogen can be formed according to the present invention which has an absorption maximum at about 544 nm.

Detailed Description Text (142):
8. Analyzer Module

Detailed Description Text (143):
A. Automated Sample Transport

Detailed Description Text (144):
The analyzer 64 may be provided with an autoloader (not shown) for automatically transporting sample tubes to the analyzer 64 for processing. Such an autoloader may include a holder which retains up to about 100 sample tubes of various sizes. A presenter which sequentially presents the sample tubes to the analyzer 64 for aspiration is operatively connected with the autoloader. A mixer which mixes the sample just before sample aspiration may also operatively associated with the autoloader. A bar code reader for reading the bar code label on each tube can also operatively be associated with the autoloader and operatively connected to the system controller to input sample information into the system controller.

Detailed Description Text (145):
B. Automated Sample Processing and Measurement

Detailed Description Text (146):
FIG. 3 illustrates a top view of one embodiment of an automated sample processing area 110 for use in the cell analysis system 60 shown in FIG. 1. The processing area 110 is part of the analyzer 64 portion of the cell analysis system 60. The processing area 110 includes a sample cup area 114 and an incubation area 118.

Detailed Description Text (148):
The reagent modules 122 include wells 128 for holding a volume of antibody reagent. In the illustrated embodiment, each reagent module 122 has a housing with a reagent well 128, preferably six in number, packaged with a particular panel of reagents. The reagents in each panel are selected so that, for the tests associated with each panel, an approximately equal amount of reagent is used from each well 128. If less than six reagents are required for the test associated with the panel, the excess wells 128 are covered by a plug (not shown). Each reagent module 122 is also fitted with a memory, such as a non-volatile RAM and the like, to store module ID and usage information. The reagent modules 122 are preferably keyed so that they may be seated in an opening (not shown), located in the thermostated block 120, in a predefined orientation. This allows the central processing unit (CPU) of the analyzer 64 to store the location and, from the usage information, the volume of the contents of each well 128 in each reagent module 122.

Detailed Description Text (184):
FIG. 5 further illustrates the analyzer's sample processing. As shown in FIG. 5, several of the sample processing cups 132, 134, 136, 138, 140 and 142 are connected to the flowcells/transducers 170, 174, 178 via a network of transport tubing 182. The RBC cup 134, RETIC cup 136, and WBC cup 138 are each in fluid communication with the impedance transducer 174 and the optical flowcell 170. The HGB cup 142 is in fluid communication with the HGB transducer 178.

Detailed Description Text (186):
In the disclosed embodiment, the analyzer module 64 is supplied with diluent, monoclonal antibody (MAb) reagents if necessary, several lysing reagents, and reticulocyte stain. The diluent, lysing reagents, and reticulocyte stain are supplied through reservoirs 192 and 196 (shown in FIGS. 7, 8 and 9) coupled to the analyzer 64. The reservoirs 192 for diluent and lysing reagents are also coupled to bulk storage containers 193. When the flow script request the filling of a reservoir, the level sensing switch (not shown) in the reservoirs 192 checks for a full condition in the reservoir, and if the instrument controller determines that the reservoir can tolerate the filling sequence at this time, a pneumatic control line 189 switches-from applying a positive pressure to applying a vacuum of about 15 inches of mercury. This vacuum causes fluid to flow from the bulk storage container 193 into the reservoir 192 until the level sensing switch senses that the reservoir 192 is full, at which time the pneumatic control line 189 returns to a positive pressure and fluid flow from the bulk storage container 193 to the reservoir 192 ceases. The Mab reagents can be supplied by disposable, pre-packaged reagent modules 122 (shown in FIGS. 3 and 4).

Detailed Description Text (187):
The analyzer 64 is provided with fluid sensors (not shown) for determining when one of the bulk containers is empty. These sensors detect air bubbles drawn into the

tubing between the bulk storage containers 193 and the reservoirs 192. The analyzer 64 informs the data station module 68 which, in turn, signals the operator about the empty container. The operator can then replace the empty container with a full one and indicate via the user interface to the data station 68 that the container has been replaced. Until the container is replaced, the analyzer 64 will not aspirate additional samples from the sample tubes, although processing of samples already begun will continue with the sufficient reagent remaining in the reservoirs.

Detailed Description Text (188):
The aspiration and dispensation by the aspiration probe 156 and the incubation probe 160 are effected by a series of piston pumps 190. FIGS. 7 and 8 illustrate how the aspiration probe 156 and incubation probe 160 are connected to piston pumps 190 and the reagent reservoirs 192. The volume and flow rate of these fluid transfers are controlled by the analyzer 64 and the data station 198.

Detailed Description Text (194):
FIG. 10a illustrates bulk transfer of sample from a sample cup 216 to the proximity of impedance transducer 174 via pump 220. FIG. 10b illustrates metered delivery of the sample by the RBC delivery syringe 204 to the impedance transducer 174. The sample cup 216 is connected to the RBC syringe 204, the impedance transducer 174 and a peristaltic pump 220 by tubing 182. A first valve 210 is placed in the tubing 182 downstream of the sample cup 216, and a second valve 212 is placed in the tubing 182 upstream of the peristaltic pump 220. The flow rate and general operation of the RBC syringe 204 are controlled automatically by the analyzer's electronics and software.

Detailed Description Text (240):
A plan view of the optics bench 350 is shown in FIG. 19. The optics bench 350 is mounted on the analyzer module 64 and includes a laser light source 352, mirrors 354, 356, lenses 358, 360, a flowcell 170 (fused-silica in an exemplary embodiment), and several detectors 400, 402, 404. The laser beam 368 is directed by a rear mirror 354, a front mirror 356, a beam adjuster 370, shaped and focussed by a pair of cylindrical lenses 358 and a laser focusing lens 360.

Detailed Description Text (258):
In a preferred embodiment of the cell analysis system 60, the pneumatic unit 72 is a separate unit having a dedicated power supply. This construction reduces weight, size and power consumption of the analyzer module 64 and data station module 68.

Detailed Description Text (260):
The vacuum pressures are controlled by the analyzer software present in a suitable memory, such as a RAM, a ROM, an EPROM, a SRAM and the like.

Detailed Description Text (263):
The data station 68 of the cell analysis system 60 has memories and other devices which apply algorithms for various cellular analyses. These algorithms are used to analyze clusters of data points generated by the analysis module 64 to yield information of clinical relevance. The disclosed integrated hematology/immunology instrument provides a single platform on which such software may be implemented, thereby providing an instrument that not only automates hematology and immunology sample processing and measurement, but also automates data analysis.

Detailed Description Text (266):
Electronic systems are found in the analyzer module 64, data station module 68, and pneumatic unit 72. The analyzer 64 provides the hardware platform for data acquisition and fluidics and motion control. In an exemplary embodiment, the data station 68 is a general purpose computer that serves as a user interface and processes, displays and stores the acquired data. The pneumatic unit 72 controls the vacuum and pressure sources.

Detailed Description Text (267):
In a preferred embodiment, the three modules are physically separate, and each unit is powered from a separate AC outlet. The data station 68 and the pneumatic unit 72 communicate with the analyzer 64 through independent serial communication channels 76, 84.

Detailed Description Text (268):
FIG. 25 is a block diagram illustrating some electronic hardware components of the analyzer 64. These components include a central processing module 500 (CPM), a data acquisition subsystem 502, and a motion control subsystem 504. The CPM 500 controls the data acquisition subsystem 502, the motion control subsystem 504, and communication functions.

Detailed Description Text (314):
The signal processing module 514 uses a 16-bit counter (not shown) to generate a time stamp with an approximately 0.5 ms resolution. The time stamp value is stored with the data from each automatic sequence iteration which resulted in valid data acquired in the ADC module 516.

Detailed Description Text (316):
FIG. 27 is a block diagram illustrating an exemplary embodiment of the motion control subsystem 504. The flow sequences and automated sample processing operations of the analyzer 64 are controlled through the motion control subsystem 504.

Detailed Description Text (317):
As illustrated, the motion control subsystem 504 includes a motor processing module 520 (MPM), a valve control module 522 (VCM), a fluid sensor module 524 (FSM), and a digital input module 526 (DIM). The MPMs 520 communicate with the CPM 500 through two independent serial links 530, 532 (500 KB), and each MPM 520 preferably controls up to 12 stepper motors 534. The VCMs 522 control all valves in the analyzer 64. The DIMs 526 monitor all digital inputs (switches, optical sensors, and magnetic sensors). The FSM 524 monitors all fluid sensors.

Detailed Description Text (320):
Software controls the major operations of the cell analysis system 60, including the analyzer flow sequences, the timing and sequence of events, gathering data, and converting measured data into meaningful results. The software is resident on suitable memories, such as RAM's, ROM's, EPROM's, SRAM's and the like, found in the system 60. The software components are preferably partitioned into the six domains (represented by circles) shown in FIG. 2.

Detailed Description Text (322):
The data station operating software 92 controls sample processing, data management, security, communications with the analyzer module and laboratory information systems (LIS), and generation of printed outputs.

Detailed Description Text (324):
The analyzer operating software (AOS) 98 controls the analyzer module's electronics (hardware), data collection, and communications to the data station module. The timing and scheduling of all analyzer activities, including the analyzer flow sequences, is also controlled by the AOS 98.

Detailed Description Text (325):
The flow sequence (FSQ) software 100 controls the mechanical components responsible for moving fluids through the analyzer module 64, including the execution of automated sample processing protocols and integrated hematology and immunology testing.

Detailed Description Text (326):
The firmware 102 includes a network of EPROM resident device controllers for various hardware modules of the analyzer 64 and pneumatic unit 72.

Detailed Description Text (327):
The operator interface (OI), data station operating software (DSOS), and algorithms use the data station module 68 as their platform. The AOS 98, FSQ software 100, and firmware 102 reside in and use the analyzer module 64 as their platform. The preferred software is a multitasking, multithreaded application.

Detailed Description Text (328):
The AOS 98 resides in the CPM 500 and is the main controller of the detailed

operation of the analyzer 64. It communicates with several slave microcontrollers responsible for stepper motor timing, analog-digital conversion, vacuum/pressure closed loop monitor/control, valve control, and digital sensor inputs. In addition, it is responsible for data, status and control communication with the data station 68 to which it is connected. The AOS 98 is preferably executed on a Motorola 68302 CPU chip. Its firmware is stored in external EPROM(s), and the downloaded AOS and flow sequences are stored in on-board RAM. An embodiment of AOS operation is shown in FIGS. 29 and 30.

Detailed Description Text (330):
Each flow sequence requires tasks of multiple analyzer components in accordance with a schedule. FIGS. 13A-F comprise a timing diagram of an exemplary flow sequence for integrating and automating hematology and immunology sample preparation and measurement on a single unit. The upper-most horizontal axis, as viewed, represents time in seconds, and the left-most vertical axis lists sample processing and measurement components of the analyzer 64. The grids of the diagram describe the activities of the analyzer components. Each of the components listed along the left vertical axis in FIGS. 13A-F performs a specific set of tasks in the flow sequence. When a component has completed its task, it begins to look for its next instruction without waiting for downstream components to finish work on the current sample.

Detailed Description Text (333):
To commence a flow sequence, the AOS 98 determines that a sample is available for aspiration. This is based either on operator activation of a pushbutton or a command from an autoloader mechanism. All the information known by the analyzer 64 about the sample is sent to the data station 68. The data station 68 responds with information about the required measurements to be performed on the sample. Based upon this response, and in conjunction with the state of the analyzer 64 (i.e. reagents, incubations, flow sequence aspiration enable/disable flags), the AOS determines whether or not to proceed with sample aspiration. Whether or not an aspiration occurs, the AOS informs the data station 68 of the status of the sample.

Detailed Description Text (336):
Upon data station request, the AOS supplies the current incubation status of all sites in the analyzer 64. This information includes incubation time, site status (clean/dirty) and site usage counts.

Detailed Description Text (339):
The flow sequence interpreter allows flow sequences to initiate event count and data collection intervals. Data generated during the data collection interval is automatically sent to the data station 68 by the AOS. The data sent to the data station 68 preferably includes at least the sample identifier, hardware counters, list mode data, and incubation time (if any). Count types preferably include:

Detailed Description Text (343):
The AOS allows the analyzer 64 to overlap counting activity on the flowcells/transducers 170, 174, 178. Thus, multiplexing and piplining the analyzer activity maximizes instrument throughput.

Detailed Description Text (344):
The analyzer 64 may be connected to external containers for waste (not shown) or bulk reagent storage (193). AOS monitors sensors that detect when the waste container becomes full or a bulk reagent storage container 193 becomes empty. Further aspiration of samples is inhibited by the AOS 98 until the condition is remedied.

Detailed Description Text (354):
The antibody reagent modules 122 are read as part of normal analyzer initialization. Thereafter, any operation that affects the status of the module 122 is recorded in the module's memory.

Detailed Description Text (355):
The AOS 98 communicates with the motor processor modules 520 which are responsible for controlling the analyzer stepper motors 534. The AOS resets the motor processor modules 520 at initialization. The AOS keeps track of the position of each motor in

the analyzer 64 and verifies this information with the controlling motor processor module 520. Position discrepancies are reported to the data station 68.

Detailed Description Text (356):
Upon successful completion of power-on self tests, the analyzer 64 accepts AOS operating software downloaded from the data station 68. At the completion of the software download, a start address is supplied from the data station 68 specifying the address at which to begin execution.

Detailed Description Text (360):
The automated sample processing protocol of the cell analysis system 60 can be considered in three phases--sample preparation, sample measurement, and sample analysis. The particular protocol for each of these phases is test dependent. For example, the preparation, measurement, and analysis for the WBC differential is different from that for platelets, reticulocytes, lymphocyte subsets, etc. General steps, however, are common to each phase.

Detailed Description Text (361):
In the first phase, automated sample preparation, the analyzer 64 aspirates a volume of the sample, transports the sample to designated cups, and mixes the sample with diluent and/or reagent as required to prepare the sample for measurement. The preparation may only involve diluting the sample, and the diluting means may also be the lysis for removing RBCs. Sometimes, as in the reticulocyte test, the preparation phase involves two steps, a first step predilution with a diluent/sheath reagent, and a second step dilution adding a known volume of fluorescent stain.

Detailed Description Text (366):
The testing protocol for the sample preparation and measurement phases of sample processing are implemented automatically by means of flow sequences, which vary in complexity. In tests involving extended incubation, the flow sequence integrates the incubation and non-incubation testing so that whenever a sample is incubating, the analyzer 64 is allowed to proceed with subsequent tests. When the incubating sample is ready for measurement, processing of further samples is interrupted and the incubated sample undergoes measurement and analysis.

Detailed Description Text (379):
The diluted sample from the RBC cup 134 is also transferred to the optical transducer by valves 236 and 238, pump 232, and syringe 240, 206. The platelets are determined in two dimensional feature space using the PSS (polarized side scatter) and IAS (intermediate angle scatter) optical parameters. The pulses from detectors 384 and 402 are processed, digitized, and stored in list mode files for processing by algorithms. The sample flow rate for measuring platelets is about 2.5 microliters per second, and the counting time through the flowcell is about 6 seconds for normal patients. This counting time is extended automatically for low count samples to improve the count statistics. The count reported from the optical transducer is platelet concentration (PLT).

Detailed Description Text (391):
When cells that meet the triple threshold criteria pass through the illuminated volume, pulses are generated at detectors 382, 384, 400, 402, and 404. The amplitudes of these pulses are filtered, amplified, digitized, and stored in list mode in the corresponding five dimensional feature space of ALL, IAS, FL3, PSS (polarized side scatter), and DSS (depolarized side scatter). The normal counting time through flowcell 170 is about 10 seconds. At the flow rate and dilution ratio described, and with a normal patient WBC count of about 7000 cells per microliter of blood volume, the resulting event count rate would be about 5000. In low count samples, this counting time can be automatically extended in order to improve the statistical accuracy of the measurement. At the conclusion of the measurement time, the sample stream is piped to waste, and probe 156 is cleaned and dried and prepared to process a subsequent sample.

Detailed Description Text (397):
The measurement process begins as the cell stream intersects the laser illuminated volume at flowcell 170. Data is acquired from optical detectors 382, 384, 400, and 402, via the system electronics and analyzer software and stored in list mode for

each Mab/blood reagent mixture. The sample has been diluted so that the cells within the stream pass through the illumination zone of the laser in single file. Each cell is detected by the presence of pulses indicative of four features--ALL(axial light loss), IAS (intermediate angle scatter), FL1(green fluorescence), and FL2(orange fluorescence). The amplitude of each pulse is amplified, digitized, and stored in list mode on the appropriate feature space axis.

Detailed Description Text (399):
The normal counting time through flowcell 170 is about 10 seconds. In certain low count samples, this counting time will be automatically extended in order to improve the counting statistics of the measurement.

Detailed Description Text (401):
The disclosed automated sample preparation features accommodate numerous antibody panels for use in a variety of immunology and phenotyping tests. For lymphocyte subsets, each panel preferably includes five 2-color antibody sets. Preferably, each antibody set includes one antibody (Mab) marked with FITC (fluorescein isothyocyanate) and the like, and a second Mab marked with PE (Phycoerithrin) and the like. The antibodies are distinguished by cluster designation (CD) numbers. Illustrating by means of example, at least the following lymphocyte subset Mabs may be included in a panel.

Detailed Description Text (403):
In certain other phenotyping Mab tests, the number of Mab pairs, N, might be 1, and hence the required sample volume would be about 50 microliters. Any combination of Mab's may be used. For some tests, the volume of Mab reagent required might be based on an estimate of the WBC patient count obtained from the hematology measurements made on the sample. As for example, in extreme cases of leukocytosis or leukopenia, it may be necessary to adjust the ratio of Mab antibody to patient blood to assure adequate antibody binding or to prevent excess free-antibody background. Because the hematology measurements do not require incubation, they proceed through the flowcell transducer well before the lymphocyte subset sample preparations are completed. The data station can therefore calculate an estimated patient count of the hematology results for that sample to enable the analyzer 64 to adjust as necessary the Mab to blood ratios in order to carry out these tests.

Detailed Description Text (406):
The reagent of the preferred embodiment contains a fluorescent dye with an excitation maximum near the 488 nm argon laser wavelength and a high quantum yield. The preferred reagent stains both DNA and RNA quickly, and in such a way that a single dimension fluorescence histogram avoids the normal WBC confusion. It is so sensitive that the analyzer 64 will detect two fragments of RNA in a cell. The method is linear to up to about 90% reticulocyte count.

Detailed Description Text (581):
22--If a peak at ALL<75 exists, the events with a PSS value greater than the PSS threshold (about 45) are classified as NRBCs and undergo no further analysis.

Detailed Description Text (675):
The validity of the lymphocyte subset measurements described in these Examples is demonstrated by comparing the analysis results using an embodiment of this invention with results of conventional manual flow cytometry assays. The results of such a comparison, between an embodiment of the current invention (termed BB3) and conventional analyses on a FACScan system by Becton Dickinson Immunocytometry Systems, are presented in FIGS. 62-D.

Other Reference Publication (6):
Cornbleet, MD., J., Spurious Results from Automated Hematology Cell Counters Laboratory Medicine vol. 14, No. 8 Aug. 1983.

CLAIMS:

1. An automated method for distinguishing and differentiating cells in a whole blood sample with an automated instrument system, the automated method performed by the automated instrument system comprising the steps of:

b. dispensing at least two aliquots of the whole blood sample into at least one sample receiving vessel on the automated instrument system, each of the at least two aliquots containing a cell;

c. analyzing each of the at least two aliquots, by passing each of the at least two aliquots through an optical flow cell on the automated instrument system and detecting at least multi-angle light scatter and fluorescence signals from at least one of the at least two aliquots;

f. analyzing the another aliquot by passing the another aliquot through an impedance transducer on the automated instrument system and detecting impedance signal from the another aliquot;

2. The automated method of claim 1 wherein a third aliquot is dispensed into the at least one sample receiving vessel and is analyzed optically with the optical flow cell for determining platelets in the whole blood sample.

3. The automated method of claim 1 wherein one of the at least two aliquots is analyzed for white blood cells and fluorescent cells or cell bodies.

4. The automated method of claim 3 wherein the fluorescent cells or cell bodies are nucleated red blood cells.

5. The automated method of claim 1 wherein a third aliquot or one of the at least two aliquots is dispensed into the at least one sample receiving vessel and is analyzed for multi-angle light scatter fluorescence with the optical flow cell.

6. The automated method of claim 5 wherein the third aliquot or one of the at least two aliquots is analyzed to collect data used to report a result comprising information about fluorescent cells or cell bodies in the whole blood sample and the cells or cell bodies are reticulocytes.

7. The automated method of claim 1 wherein the information reported about white blood cells is quantitative and is obtained from processing light scatter and fluorescent signal generated data.

8. The automated method of claim 7 wherein the quantitative white blood cell information comprises a five-part differential.

9. The automated method of claim 8 wherein the five-part differential further comprises a percentage of nucleated red blood cells.

10. The automated method of claim 1 wherein at least one of the least two aliquots is analyzed to provide information about viability of the cell in the whole blood sample.

11. An automated method for distinguishing and differentiating cells in a whole blood sample with an automated instrument system capable of performing both hematology analysis and fluorescent cytometry analysis to which a whole blood sample is provided, the automated method comprising the steps of:

(a) selecting a series of one or more tests to be performed on the whole blood sample by the automated instrument system;

(b) correlating the one or more tests to be performed on the whole blood sample by the automated instrument system;

(g) transporting a first aliquot of the diluted sample through a first flow transducer of the automated instrument system;

wherein the automated instrument system automatedly performs method steps (b) through (m) without physically separating cells from the whole blood sample or an aliquot thereof and results of hematology analysis are utilized in at least reporting of the results of the fluorescent cytometry analysis.

14. The method of claim 11 wherein the second aliquot of the dilute sample is transported by the automated instrument system through the second flow transducer and multi-angle light scatter is detected to count and to differentiate platelets in the second aliquot of the diluted sample.

16. The automated method of claim 11 wherein a reported quantitative white blood cell result is obtained from multi-angle light scatter and fluorescent detected data.

17. The automated method of claim 16 wherein the white blood cell result comprises a five-part differential.

18. The automated method of claim 17 wherein the five part differential further comprises a percentage of nucleated red blood cells.

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L5: Entry 50 of 53 File: USPT

DOCUMENT-IDENTIFIER: US 5264369 A
TITLE: Method of preparing specimen for counting subpopulations of leukocytes and erythroblasts

Abstract Text (1):
A hematological specimen for classifying and counting leukocytes with a flow cytometer is prepared. A sample to be assayed is prepared by eliminating influences of erythrocytes from a hematological sample without changing leukocytes morphologically by adding a first aqueous solution of a low osmotic pressure including a buffer for adjusting the pH value within an acidic region and a second aqueous solution including an osmolarity compensating agent and a buffer for giving pH value suitable for staining, optionally further adding a salt, which dissociates into ions in aqueous solutions so as to control the electrical conductivity of the aqueous solution at a preferable level, while damaging the cell membranes of erythroblasts contained in said sample; and staining the leukocytes with at least four dyes including Astrazon Yellow 3G and Neutral Red. Thus leukocytes contained in the hematological sample can be classified into at least eight groups including immature granulocytes, erythroblasts, basophils, eosinophils, lymphocytes, monocytes and neutrophils, or nine groups invoving one having blasts in addition to the above-mentioned eight groups, by assaying a single specimen with a flow cytometer.

Brief Summary Text (3):
The present invention relates to a process for preparing a specimen for classifying and counting blood corpuscles in the practice of clinical testing. More particularly, it relates to a method for preparing a specimen to be used in classifying and counting leukocytes with a flow cytometer by means of optical or optical/electrical measurements on blood corpuscles.

Brief Summary Text (7):
It is well known that the peripheral blood of patients with, for example, leukemia, hemolytic anemia or cancer contains immature granulocytes, blasts and erythroblasts, which are usually observed not in the peripheral blood but in the bone marrow, in addition to the above-mentioned five types. These three blood corpuscles will be called "abnormal cells" hereinafter. Therefore, it is highly important to detect, classify and count these abnormal cells for diagnostic purposes.

Brief Summary Text (10):
Therefore attempts have been made in order to automatically classify and count a number of leukocytes to thereby increase accuracy and save labor. Recently, automated devices based on a flow system for solving the above-mentioned problems have been marketed.

Brief Summary Text (11):
These automated devices may be roughly classified into the following three types depending on the measurement principle.

Brief Summary Text (21):
The above-mentioned disadvantages of the manual method are solved by each of these automated methods. From the viewpoint of precision, in particular, a remarkable improvement has been achieved. Thus these automated methods are almost satisfactory in the practice of clinical testing.

Brief Summary Text (23):

Separately, there have been reported some methods whereby fluorescence or scattered light of each leukocyte in a fluorochrome-stained blood sample are measured with a flow cytometer so as to classify leukocytes. Major examples of these methods are described in Japanese Patent Publication No. 853/1984. Japanese Patent Laid-Open No. 20820/1975 and Japanese Patent Publication No. 70166/1988.

Brief Summary Text (24):
When a specimen, obtained by eliminating influences of blood corpuscles other than leukocytes from a hematological sample by an appropriate method, is assayed with a marketed flow cytometer as shown in FIG. 1, it is generally known that a scattergram as shown in FIG. 3 is obtained and the leukocytes are divided into three subpopulations respectively comprising lymphocytes 1', monocytes 2' and granulocytes 3' mainly depending on the difference in the side scattered light intensity and each of these subpopulations can be easily classified and counted.

Brief Summary Text (25):
It is also possible, further, to divide the granulocytes into subpopulations comprising eosinophilis, basophils and neutrophils by combining the said process with the above-mentioned fluorochrome-staining. In Japanese Patent Laid-Open No. 134958/1988, we have already disclosed a method of dividing leukocytes into five subpopulations and classifying and counting each subpopulation with the use of a flow cytometer and reagents to be used in this method.

Brief Summary Text (27):
On the other hand, U.S. Pat. No. 4,500,509 discloses a manual method for classifying and counting leukocytes wherein all leukocytes including abnormal cell are fluorochrome-stained with Basic Orange 21 and then treated under a fluorescent microscope. However the above-mentioned disadvantages of the manual method cannot be solved by this method. Thus this U.S. patent provides no automated method.

Brief Summary Text (29):
As described above, the present invention aims at specifically detecting, classifying and counting abnormal cells, which cannot be achieved by conventional automated methods, and providing a method for preparing a specimen for flow cytometry in order to classify and count abnormal cells and to classify and count leukocytes involving abnormal cells.

Drawing Description Text (2):
FIG. 1 is a schematic view showing the construction of a common flow cytometer.

Drawing Description Text (3):
FIG. 2 is a schematic view of a flow cytometer to be used in the method of the present invention by which optical signals and electric resistance signals can be measured simultaneously.

Drawing Description Text (4):
FIG. 3 is a scattergram obtained by measuring a specimen, which is prepared by eliminating influences of blood corpuscles other than leukocytes from a hematological sample, with the flow cytometer shown in FIG. 1.

Detailed Description Text (40):
In general, a hematological sample contains about 1,000 times as many erythrocytes as leukocytes. In flow cytometry, the intensity of a scattered light signal of lymphocytes is comparable to that of erythrocytes, which makes it difficult to separate lymphocytes from erythrocytes. As a result, accurate classification data of leukocytes can hardly be obtained. When a large number of erythrocytes pass through a detection unit of a flow cytometer simultaneously with leukocytes, furthermore, the scattered light signal of the leukocytes becomes less accurate and, therefore, it becomes difficult to separate lymphocytes, monocytes and neutrophils depending on side scattered light intensity. Alternately, cell volume cannot be measured with a device of the electrical resistance assay system in the presence of a large amount of erythrocytes. In order to solve these problems, it is required to eliminate erythrocytes in a hematological sample by some method.

Detailed Description Text (45):

In usual measurement of optical parameters with a flow cytometer, it is not needed to control the electrical conductivity of a prepared specimen. When cell volume is to be measured based on the electrical resistance assay principle, however, it is required to adjust the electrical conductivity of a specimen to a level suitable for the measurement of the cell volume by the electrical resistance assay system. This can be achieved by adding an appropriate amount of salts which dissociate into ions in aqueous solutions.

Detailed Description Text (51):
A flow cytometer is a device by which at least three optical data (red fluorescence, green fluorescence, side scattered light), preferably four optical data (forward scattered light and the above-mentioned three factors) can be measured, as shown in FIG. 1. It is further preferable to use a flow cytometer as provided with an electrical resistance assay system (refer to FIG. 2) by which cell volume can be simultaneously measured.

Detailed Description Text (70):
The amount sufficient for staining the nuclei of cells with damaged cell membranes means such an amount sufficient for emitting fluorescence of an intensity by which erythroblasts can be separated from other cells in flow cytometry. The optimum concentration varies from dye to dye and thus should be determined through experiment. In the case of ethidium bromide, for example, a concentration of 10 mg/l or above is suitable.

Detailed Description Text (89):
The amount sufficient for staining either or both of the nuclei and cytoplasm of leukocytes means such an amount sufficient for emitting fluorescence of an intensity by which leukocytes can be separated from other cells in flow cytometry. The optimum concentration varies from dye to dye and thus should be determined through an experiment. In the case of Astrazon Orange R, for example, a concentration of 100 mg/l or above is suitable. The effects of these 16 dyes have been experimentally confirmed by us. However the present invention is not restricted thereto and any dye may be used so long as it satisfies the above-mentioned requirements.

Detailed Description Text (93):
The mixing ratio by volume of the hematological sample to the first aqueous solution is not particularly restricted. In the measurement with a flow cytometer, a mixing ratio ranging from 1:5 to 1:200 is preferable.

Detailed Description Text (97):
In order to limit damage to leukocytes and maintain at least lymphocytes, monocytes and neutrophils in a shape required for separation depending on scattered light, it is advantageous that the osmotic pressure of the mixture ranges from 100 to 500 mOsm/kg, still preferably from 200 to 400 mOsm/kg. When the osmotic pressure of the mixture does not fall within this range, it is recommended to add an osmolarity compensating agent to the aqueous solution. The type of the osmolarity compensating agent is not particularly restricted. It is preferable to use substances commonly employed for maintaining biological cells at physiological osmotic pressure (for example, alkali metals and saccharides) therefor. When cell volume is to be measured with a flow cytometer provided with an electrical resistance assay system, it is preferable to control the electrical conductivity of the finally prepared specimen. It is generally advantageous to adjust the electrical conductivity of said specimen to the same level as that of the sheath fluid.

Detailed Description Text (102):
Now, a flow cytometer to be used in the embodiment of the present invention will be illustrated. FIG. 1 is a schematic diagram showing the construction of a common flow cytometer. In FIG. 1, 1 is a light source of the flow cytometer from which light of a wavelength suitable for exciting the specific fluorescence at least from eosinophils, basophils and immature granulocytes stained with Astrazon Yellow 3G and Neutral Red is emitted. As this light source 1, an argon ion laser or a mercury arc lamp capable of emitting light of 400 to 520 nm in wavelength may be preferably used. The light from the light source is condensed in a flow area 20 of particles by a lens 2 in the form of a flat circle and a particle 13 (cell etc.) passing therethrough is irradiated therewith. Thus forward scattered light 21 is emitted

forward from the particle 13, while red fluorescence 22, green fluorescence 23 and side scattered light 24 are emitted sideways from the same.

Detailed Description Text (109):
The term "forward scattered light" to be used herein means scattered light emitted from a cell passing through the detection unit at a narrow angle of almost 0.degree. based on the emission axis of the light source. The term "side scattered light" as used herein means scattered light emitted from a cell to be detected at an angle of almost 90.degree. based on the emission axis of the light source. The term "red fluorescence" means fluorescence of a wavelength of 560 nm and above from among those emitted in all directions from a cell. Fluorescence at almost 0.degree. or 90.degree. from the emission axis of a light source can be condensed with a usual flow cytometer.

Detailed Description Text (110):
The term "green fluorescence" means fluorescence of a wavelength around 520 to 560 nm from among those emitted in all directions from a cell. Fluorescence at almost 0.degree. or 90.degree. from the emission axis of a light source can be condensed with a usual flow cytometer.

Detailed Description Text (111):
FIG. 2 is a schematic diagram of a flow cytometer to be used in the present invention by which optical signals and electrical signals can be simultaneously measured. Optical signals can be detected by the same method as the one described regarding FIG. 1. On the other hand, electrical signals can be detected as follows. A flow cell 18 is provided with an orifice 18a for measuring electrical resistance. Light emitted from the light source 1 is condensed around the center of the orifice 18a with a lens 2. As is well known, the accurate volume of a cell of a certain size can be determined by measuring a change in electrical resistance between electrodes 20a and 20b due to the passage of the cell through the orifice 18a. In the present invention, the electrical signals and optical signals can be simultaneously detected.

Detailed Description Text (118):
0.90 ml of the first reagent solution of the above Composition Example 1 was mixed with 0.05 ml of peripheral blood containing abnormal cells (erythroblasts and immature granulocytes) and was then allowed to incubate for 5 seconds or longer. Then 0.01 ml of the second reagent solution was further added thereto and the obtained mixture was allowed to incubate for an additional 10 seconds or longer. Thus a speciment to be assayed was obtained. Leukocytes were classified and counted by measuring the red fluorescence, green fluorescence, side scattered light and forward scattered light of each cell with a flow cytometer of FIG. 1. Then a scattergram was formed by referring the intensity of red fluorescence and that of green fluorescence to as the coordinate axes, as shown in FIG. 4. Thus leukocytes were divided into subpopulations, namely, one comprising erythroblasts [NRBC], one comprising eosinophils [Eo], one comprising other leukocytes [A1] and one comprising blood corpuscles other than leukocytes [A2]. Then the whole leukocytes were delineated within a window 1 [W1] and counted. Thus the total leukocyte number was determined. Next, the eosinophils and the erythroblasts were gated respectively with a window 2 [W2] and a window 3 [W3], followed by counting. Other leukocytes were taken out with a window 4 [W4] and a scattergram was formed by referring the side scattered light intensity and the intensity of green or red fluorescence as to the coordinate axes, as shown in FIG. 5. Thus subpopulations involving one comprising lymphocytes [Lym], one comprising monocytes [Mono], one comprising neutrophils [Neut], one comprising immature granulocytes 1 [Im1] and one comprising immature granulocytes 2 [Im2] were obtained. Each of these subpopulations was gated with a window and counted. The value thus obtained was divided with the total leukocyte number determined above. Thus the percentage of each leukocyte type was obtained.

Detailed Description Text (121):
0.90 ml of the first reagent solution of the above Composition Example 2 was mixed with 0.05 ml of peripheral blood and then allowed to incubate for 5 seconds or longer. Then 0.10 ml of the second reagent solution was further added thereto and the obtained mixture was allowed to incubate for an additional 10 seconds or longer. Thus a specimen to be assayed was obtained. Leukocytes were classified and counted

by measuring the red fluorescence, green fluorescence, side scattered light and cell volume of each cell with a flow cytometer provided with an electrical resistance assay system of FIG. 2. Then a scattergram was formed by referring the intensity of red fluorescence and that of green fluorescence to as the coordinate axes, as shown in FIG. 6. Thus leukocytes were divided into subpopulations, namely, one comprising erythroblasts [NRBC], one comprising eosinophils [Eo], one comprising other leukocytes [A3]. Then the whole leukocytes were delineated within a window 5 [W5] and counted. Thus the total leukocyte number was determined. Next, the eosinophils and the erythroblasts were gated respectively with a window 6 [W6] and a window 7 [W7], followed by counting. Other leukocytes were taken out with a window 8 [W8] and a scattergram was formed by referring the side scattered light intensity and the intensity of green or red fluorescence as to the coordinate axes, as shown in FIG. 7. Thus subpopulations involving one comprising lymphocytes and blasts [Lym+Blast], one comprising monocytes [Mono], one comprising neutrophils [Neut], one comprising basophils [Ba], one comprising immature granulocytes 1 [Im1] and one comprising immature granulocytes 2 [Im2] were obtained. Each of these subpopulations was gated with a window and counted. The lymphocytes and blasts were gated with a window 9 [W9] and a scattergram was formed by referring the side scattered light intensity and the cell volume, measured based on the electrical resistance assay principle, to as the coordinate axes, as shown in FIG. 8. Thus two subpopulations, namely, one comprising lymphocytes [Lym] and one comprising blasts [Blast] were observed. Each subpopulation was delineated within a window and counted. The value thus obtained was divided with the total leukocyte number determined above. Thus the percentage of each leukocyte type was obtained.

Detailed Description Text (122):
1. A hematological sample is treated by the method of the present invention and thus a specimen to be assayed by flow cytometry is prepared. Thus immature granulocytes can be specifically stained and separated.

Detailed Description Text (123):
As a result, leukocytes can be divided into at least eight groups simply by measuring a single specimen with a flow cytometer.

Detailed Description Text (124):
2. The measurement with a flow cytometer provided with an electrical resistance assay system further makes it possible to separate blasts.

Detailed Description Text (125):
Thus leukocytes can be divided into at least nine groups simply by measuring a single specimen with a flow cytometer.

CLAIMS:

1. A method for preparing a specimen for classifying and counting blood corpuscles into at least eight groups, namely, two comprising immature granulocytes group 1 and immature granulocytes group 2, one comprising erythroblasts, one comprising basophils, one comprising eosinophils, one comprising lymphocytes, one comprising monocytes and one comprising neutrophils, by assaying a single specimen with a flow cytometer, which comprises the following steps:

4. A method for preparing a specimen for classifying and counting blood corpuscles into at least nine groups, namely, two comprising immature granulocytes group 1 and immature granulocytes group 2, one comprising erythroblasts, one comprising blasts, one comprising basophils, one comprising eosinophils, one comprising lymphocytes, one comprising monocytes and one comprising neutrophils, by assaying a single specimen with a flow cytometer, which comprises the following steps:

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DOCUMENT-IDENTIFIER: US 5917584 A
TITLE: Method for differentiation of nucleated red blood cells

Abstract Text (1):
A method is provided for differentiation of nucleated red blood cells. In addition, the method provides for a concurrent differentiation of leukocytes in a blood cell sample by suitable electronic and optical measurements. The method includes exposing a blood cell sample to a reagent system to lyse mature red blood cells and subsequently analyzing nucleated red blood cells in a flow cell by optical analysis. A concurrent differentiation of nucleated blood cells and leukocytes can be performed using electronic and optical analysis. The electronic and optical analysis includes light scatter and impedance measurements. This method eliminates the use of nuclear stain for identification of nucleated red blood cells. The method of the present invention, for the first time, reports differentiation and enumeration of nucleated red blood cells without using fluorescence.

Brief Summary Text (4):
Normal peripheral blood contains mature red blood cells which are free of nucleus and reticulum. Nucleated red blood cells (NRBCs), or erythroblasts, are immature red blood cells. They normally occur in the bone marrow but not in peripheral blood. However, in certain diseases such as anemia and leukemia, NRBCs also occur in peripheral blood. Therefore, it is of clinical importance to measure NRBC. Traditionally, differentiation and enumeration of NRBC are performed manually. The process involves the smearing of a blood sample on a microscope slide and staining the slide, followed by manual visual analysis of the individual slide. The NRBC concentration is reported as number of NRBCs per 100 white blood cells. Usually, 200 white blood cells and the number of NRBCs present in the same region on a blood smear are counted and the numbers are divided by two to express the NRBC concentration as the number of NRBCs/100 white blood cells. This approach is extremely time-consuming as well as being subjective to the interpretation of the individual analyzing the slide.

Brief Summary Text (5):
In recent years, several fluorescence flow cytometry methods have been developed for differentiating NRBCs. These methods utilize specific nuclear staining technique to distinguish NRBCs from other cell populations because it is not easy to differentiate NRBCs based on their electronic or optical properties.

Brief Summary Text (7):
U.S. Pat. No. 5,559,037 (to Kim et al.) discloses a method for flow cytometric analysis of NRBCs and leukocytes. The method comprises lysis of red blood cells and NRBC cytoplasm from a whole blood sample to expose the NRBC nuclei to a vital nuclear stain and minimizing the permeation of the vital nuclear stain into the leukocytes and analyzing the sample by measuring fluorescence and two angles of light scatter. This method features a triple triggering method which blocks the signals from debris (fluorescent and non-fluorescent) and identifies the signals which fall below the ALL trigger but above the fluorescence trigger (FL3) as NRBCs. ALL is the axial loss of light or the light scatter signals detected at 0.degree. from the incident light. Therefore, pre-gating of signals in more than one dimension is required in this method for identification of NRBC population. Since leukocytes are also nucleated cells, staining of these cells needs to be prevented to avoid interference to the fluorescence measurement. The preservation of leukocyte membrane and minimizing the permeation of the nuclear stain into the leukocytes are achieved

by concurrently fixing the leukocytes with an aliphatic aldehyde during lysis of red blood cells. The aldehyde fixatives are known as hazardous chemicals. In addition, the method requires heating of the reagent to 42.degree. C. in order to obtain the NRBC and leukocyte differentiations.

Brief Summary Text (8):
U.S. Pat. No. 5,631,165 (to Chupp et al.) discloses an automated method for counting and differentiating nucleated red blood cells or reticulocytes and cell surface antigens in a lysed blood sample. The method detects multi-angle light scatter and fluorescence signals from the lysed blood sample in a flow cell. Similar to U.S. Pat. No. 5,559,037 (to Kim et al.), this method requires detection of fluorescence signals from nucleated red blood cells or reticulocytes produced by nuclear staining with a fluorescent dye.

Brief Summary Text (9):
The above-described methods are able to differentiate and enumerate NRBCs and leukocytes by fluorescence flow cytometry. However, fluorescence measurement is a complex and expensive detection method.

Brief Summary Text (10):
Current automated hematology analyzers, such as Abbott Cell-Dyn.RTM. 3500, COULTER.RTM. STKS.RTM., Technicon H*1.RTM. and TOA Sysmex.TM. NE-8000 are only able to provide NRBC flagging for the possible presence of NRBCs in an analyzed blood sample when the instruments sense an increased amount of signals near red blood cell debris area of a histogram. However, such techniques frequently generate false positive flagging because many other blood abnormalities can cause increased signals at the same area, such as platelet clumps and sickle cells, as well as red cell debris from insufficiently lysed blood samples. In these methods NRBCs are not identified. Instead, only a common NRBC sample distribution pattern in a histogram or a dotplot is recognized by the instrument which can be easily confused with a similar pattern generated by above-mentioned other causes. For the flagged samples, including false positive flags, re-examination of the sample with manual method is required in clinical laboratories. Another problem with the NRBC containing samples is that the white blood cell count (WBC) reported by hematology analyzers is not accurate for these samples since NRBCs could elevate the WBC by being misidentified as white cells. On the other hand, analysis of leukocyte populations from whole blood samples is an integral part of diagnostic procedures regarding a multiplicity of pathologies. The ability to analyze the major subpopulations of leukocytes in an automated manner is essential for a rapid diagnosis of a single blood sample and for the rapid processing of many samples at once.

Brief Summary Text (11):
U.S. Pat. No. 5,155,044 (to Ledis et al.) discloses a method for isolation and analysis of leukocytes from a whole blood sample, which enables differentiation of leukocytes into five subpopulations in a one-step measurement on an automated hematology analyzer. The detection technique involves a concurrent light scatter measurement and impedance measurements in both DC (direct current) and RF (radio frequency). This method is simple and fast, but it does not provide differentiation of NRBCs.

Brief Summary Text (13):
U.S. Pat. No. 5,686,308 (to Li et al.) describes a lysing reagent system and a method for differentiation of leukocytes into five subpopulations in a one-step measurement on an automated hematology analyzer. The lytic reagent system comprises a lytic reagent comprising an ethoxylated long chain amine compound and acid to adjust the pH of the lytic reagent to be within the range of 2.0 to 3.6; and a hypertonic, alkaline stabilizing reagent. This patent teaches a reagent and method for differentiation of leukocyte subpopulations, but does not teach differentiation of nucleated red blood cells.

Brief Summary Text (16):
One object of the present invention is to provide a method which permits the differentiation of nucleated red blood cells on an automated hematology analyzer without using fluorescence or nuclear stain. The method comprises providing a blood sample for analysis; lysing mature red blood cells from said blood sample; analyzing

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     (FILE 'HOME' ENTERED AT 16:53:19 ON 15 JUL 2002)

     FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 16:54:31
     ON 15 JUL 2002
L1        29752 S ERYTHROBLAST? OR NRBC? OR ((NUCLEATED OR NUCLEUS) (2A) ((RED
L2           39 S L1 (P) ((MATUR? OR STAGE?) (5A) (CLASS?))
L3           20 DUP REM L2 (19 DUPLICATES REMOVED)
L4         1385 S L1 AND (CYTOMET? OR FACS OR (FLOW (3A) SORT?))
L5          782 S L1 (6P) (CYTOMET? OR FACS OR (FLOW (3A) SORT?))
L6           41 S L1 (3P) ((MATUR? OR STAGE?) (5A) (CLASS?))
L7            4 S L5 (6P) L6
L8            4 S L5 AND L6
L9            4 DUP REM L7 (0 DUPLICATES REMOVED)
L10           7 S L6 AND (AUTOMAT? OR ANALYZER?)
L11           6 DUP REM L10 (1 DUPLICATE REMOVED)

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L3 ANSWER 3 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2002:151350 BIOSIS
DOCUMENT NUMBER: PREV200200151350

TITLE: Preliminary observation on various disorders in erythropoiesis with automated hematology analyzer, XE-2100 and its potential clinical applications.

AUTHOR(S): Wang, Fu-sheng (1); Bartels, Piet; Schoorl, Margreet; Hamaguchi, Yukio; Versteege, Paul
CORPORATE SOURCE: (1) R and D, Sysmex Corporation of America, Long Grove, IL USA

SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 13b. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Since 1997 we have established automated methods for counting **nucleated red blood cells** (**NRBC**) on a hematology analyzer Sysmex XE-2100, studied **NRBC** staging, and are developing **NRBC** quality control/calibration materials for **NRBC** analysis. **NRBC** counting on XE-2100 has revealed very good correlation with cell morphology and flow cytometry reference methods. **NRBCs** are **classified** into three **stages** based on changes of cell biology and structure. Quality control and calibration materials prepared with human blood containing **NRBCs** demonstrated stability for at least 50 days. We have also observed various disorders in erythropoiesis with Sysmex XE-2100. We were attracted by the reported scattergram from the **NRBC** channel of this hematology analyzer in cases with sickle cell anemia. The **NRBCs** are depicted in a narrow area concordant with characteristics of small size and low fluorescent intensity. The morphology studies revealed small **NRBCs** with condensed small nuclei. Further observation indicated that this phenomenon also existed in other congenital hemolytic diseases such as thalassemia, and iron deficiency. For comparison, we have investigated peripheral blood **NRBCs** in cases of subjects with respiratory failure. In most of these cases, two or more **NRBC** populations with different cell sizes and fluorescent intensities are detected. Similarly, the morphology showed **NRBCs** in different maturation stages. Recently we evaluated a case of M6 leukemia. Morphologically, the majority (more than 80%) of **erythroblasts** were pro-**erythroblasts**. The Sysmex XE-2100 test methodology showed a **NRBC** population with very strong fluorescent intensity. The observations on these disorders in erythropoiesis with the hematology analyzer demonstrated: 1) Based on cell size and fluorescent intensity, **erythroblasts** in different stages occupy separate positions in the **NRBC** scattergram. This observation can be used for **NRBC** staging and future automated bone marrow differential count, 2) The different behaviors in cases of various erythropoietic disorders on the automated hematology analyzer can be used for disease screening and differential diagnosis. Because of low costs and high sensitivity for detection of different **erythroblast** populations, the hematology analyzer can be used to evaluate clinical course and efficacy of clinical therapy.

L9 ANSWER 1 OF 4 USPATFULL

ACCESSION NUMBER: 2001:67463 USPATFULL

TITLE: Method and apparatus for analyzing cells in a whole blood sample

INVENTOR(S): Rodriguez, Carlos M., Miami, FL, United States
Cano, Jose M., Miami, FL, United States
Carrillo, Barbara, Miami, FL, United States
Gordon, Kristie M., Coral Gables, FL, United States
Horton, Allan F., Miami, FL, United States
Paul, Ronald D., Fort Lauderdale, FL, United States
Wells, Mark A., Davie, FL, United States
Wyatt, James L., Plantation, FL, United States

PATENT ASSIGNEE(S): Coulter International Corp., Miami, FL, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6228652	B1	20010508	
APPLICATION INFO.:	US 1999-251019		19990216	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wallenhorst, Maureen M.
LEGAL REPRESENTATIVE: Kurz, Warren W., Alter, Mitchell E.
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 11
NUMBER OF DRAWINGS: 39 Drawing Figure(s); 25 Drawing Page(s)
LINE COUNT: 1348

AB A blood analyzing instrument includes a single transducer for simultaneously measuring the DC volume, RF conductivity, light scattering and fluorescence characteristics of blood cells passing through a cell-interrogation zone. Preferably, the transducer includes an electro-optical flow cell which defines a cell-interrogation zone having a square transverse cross-section measuring approximately 50.times.50 microns, and having a length, measured in the direction of cell flow, of approximately 65 microns.

SUMM . . . blood cells are three basic types, namely, red cells (erythrocytes), white cells (leukocytes), and platelets. Depending on the level of **maturity**, red cells are often further **classified** into three subsets, namely, **nucleated red blood cells** (**NRBC**'s), reticulated red cells ("retics"), and mature red blood cells (RBC's). Mature white cells, on the other hand, fall into one. . .

SUMM . . . blood-smear under a microscope. Alternatively, some blood types of an extended differential measurement can be detected using a conventional flow **cytometer**. In such an instrument, a blood sample that has been previously prepared, e.g., by either (1) mixing the sample with. . . cell is detected and used to differentiated the cells of interest from other cells in the sample. Commercial, stand-alone, flow **cytometers** are made by Beckman Coulter, Toa Medical Electronics, Cytomation, Bio-Rad, and Becton Dickinson. It is known in the prior art to integrate flow **cytometers** and hematology instruments into a single automated laboratory system in which blood samples are automatically advanced along a track past. . . a blood sample is aspirated from each vial and analyzed by the instrument. Instrument systems combining discrete hematology and flow **cytometry** instruments are commercially available from Beckman Coulter and Toa Medical Electronics, reference being made to Toa's HST Series.

SUMM . . . U.S. Pat. Nos. 5,631,165 and 5,565,499, an attempt is made to fully integrate the respective functions of hematology and flow **cytometry** instruments into a single instrument. Such an instrument comprises a plurality of transducers, including an optical flow cell adapted to. . .

L9 ANSWER 3 OF 4 USPATFULL

ACCESSION NUMBER: 97:61562 USPATFULL

TITLE: Method of using a multi-purpose beagent for subclassification of nucleated blood cells

INVENTOR(S): Kim, Young Ran, Sunnyvale, CA, United States
Kantor, Johanna, Palo Alto, CA, United States
Gill, James E., Mountain View, CA, United States
Luptovic, Sue E., San Jose, CA, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5648225		19970715
APPLICATION INFO.:	US 1994-296379		19940825 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1993-23042, filed on 25 Feb 1993, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Hutzell, Paula K.

ASSISTANT EXAMINER: Freed, Rachel Heather

LEGAL REPRESENTATIVE: Poulos, Nicholas A.

NUMBER OF CLAIMS: 10

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 16 Drawing Figure(s); 16 Drawing Page(s)

LINE COUNT: 870

AB A multipurpose reagent system for rapid analysis of a whole blood sample allowing the determination of at least five classes of peripheral white blood cells, nucleated red blood cells, and lymphocyte immunophenotyping on automated hematology instrumentation. The multipurpose reagent system lyses red cells rapidly, while it concurrently fixes white cells and preserves surface antigens on lymphocytes. The multipurpose reagent system comprises from about 3 to 7 grams per liter of a non-quaternary ammonium salt, from about 0.04 to about 0.10 percent by volume of an aliphatic aldehyde with one to four carbons, from about 10 mM to about 20 mM of a non-phosphate buffer which is inert to the aliphatic aldehyde, and a sufficient amount of water to give a pH between 5.5 and 7.5 and an osmolality of between about 160 to about 310 mOsm per liter.

SUMM . . . rapidly lysing red cells and concurrently fixing white cells, useful for performing white cell differential analyses and quantitative analyses of **nucleated red blood cells** or lymphocyte subclassification using immunophenotyping techniques on an automated clinical hematology analyzer or flow **cytometer**.

SUMM The peripheral blood of a normal subject contains red blood cells, also known as erythrocytes, and five major **classes** of **mature** white cells, also known as leukocytes. There are at least five classes of leukocytes, known as neutrophils, eosinophils, monocytes, lymphocytes. . .

SUMM Recent advances in cellular immunology and flow **cytometry** are being utilized to identify and quantify lymphocyte subclasses such as helper T cells. Lymphocyte subclassification has become an important. . . with fluorochrome-labeled monoclonal antibodies directed to specific lymphocyte surface antigens; and (3) the analysis of lymphocyte-antibody reaction products using flow **cytometry**. Currently, a great deal of effort is being directed towards the development of whole blood methods that bypass the need. . .

SUMM Generally speaking, the reagent systems available for use during the analysis of **nucleated red blood cells** (**NRBC**) are as yet unable to allow for the differentiation and counting of **NRBC** signals from red cell stroma or large platelets and only allow the instrument to flag possible **NRBC** signals.

SUMM . . . in an automated clinical analyzer. For example, the Tris buffered ammonium chloride solution recommended by K. A. Murihead in Clinical **Cytometry**, Ann. N.Y. Acd. Sci., vol. 468, pp. 113-127 (1986) takes 5 to 10 minutes to lyse red cells, which is. . .

ER: 96:41135 USPATFULL
TITLE: Multipurpose reagent system for rapid lysis of whole blood
INVENTOR(S): Kim, Young R., Sunnyvale, CA, United States
Kantor, Johanna, Palo Alto, CA, United States
Gill, James E., Mountain View, CA, United States
Luptovic, Sue E., San Jose, CA, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5516695		19960514
APPLICATION INFO.:	US 1994-297662		19940829 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-23042, filed on 25 Feb 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wityshyn, Michael G.
ASSISTANT EXAMINER: Freed, Rachel Heather
LEGAL REPRESENTATIVE: Schmidt, Richard D.
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 910
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A multipurpose reagent system for rapid analysis of a whole blood sample allowing the determination of at least five classes of peripheral white blood cells, nucleated red blood cells, and lymphocyte immunophenotyping on automated hematology instrumentation. The multipurpose reagent system lyses red cells rapidly, while it concurrently fixes white cells and preserves surface antigens on lymphocytes. The multipurpose reagent system comprises from about 3 to 7 grams per liter of a non-quaternary ammonium salt, from about 0.04 to about 0.10 percent by volume of an aliphatic aldehyde with one to four carbons, from about 10 mM to about 20 mM of a non-phosphate buffer which is inert to the aliphatic aldehyde, and a sufficient amount of water to give a pH between 5.5 and 7.5 and an osmolality of between about 160 to about 310 mOsm per liter.

SUMM . . . . rapidly lysing red cells and concurrently fixing white cells, useful for performing white cell differential analyses and quantitative analyses of **nucleated red blood cells** or lymphocyte subclassification using immunophenotyping techniques on an automated clinical hematology analyzer or flow **cytometer**.

SUMM The peripheral blood of a normal subject contains red blood cells, also known as erythrocytes, and five major **classes** of **mature** white cells, also known as leukocytes. There are at least five classes of leukocytes, known as neutrophils, eosinophils, monocytes, lymphocytes. . . .

SUMM Recent advances in cellular immunology and flow **cytometry** are being utilized to identify and quantify lymphocyte subclasses such as helper T cells. Lymphocyte subclassification has become an important. . . . with fluorochrome-labeled monoclonal antibodies directed to specific lymphocyte surface antigens; and (3) the analysis of lymphocyte-antibody reaction products using flow **cytometry**. Currently, a great deal of effort is being directed towards the development of whole blood methods that bypass the need. . . .

SUMM Generally speaking, the reagent systems available for use during the analysis of **nucleated red blood cells** (**NRBC**) are as yet unable to allow for the differentiation and counting of **NRBC** signals from red cell stroma or large platelets and only allow the instrument to flag possible **NRBC** signals.

SUMM . . . . in an automated clinical analyzer. For example, the Tris

buffered ammonium chloride solution recommended by K. A. Murihead in Clinical **Cytometry**, Ann. N.Y. Acd. Sci., vol. 468, pp. 113-127 (1986) takes 5 to 10 minutes to lyse red cells, which is. . .

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L11 ANSWER 6 OF 6 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 87298858 MEDLINE

DOCUMENT NUMBER: 87298858 PubMed ID: 3620064

TITLE: A quantitative analysis of the human bone marrow erythroblastic cell lineage using the SAMBA 200 cell image processor. I. The normal maturation sequence.

AUTHOR: Gauvain C; Seigneurin D; Brugal G

SOURCE: ANALYTICAL AND QUANTITATIVE CYTOLOGY AND HISTOLOGY, (1987 Jun) 9 (3) 253-62.
Journal code: 8506819. ISSN: 0884-6812.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198709

ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870929

AB A quantitative image analysis of the normal maturation sequence for the human bone marrow **erythroblastic** lineage was performed using the SAMBA 200 cell image processor. The different image analysis steps (image acquisition, preprocessing, segmentation, parametrization and data analysis) are briefly described. Thirty-three parameters related to geometry, color, texture and densitometry were computed on 638 cell images belonging to the five **erythroblastic maturation stages**. The **automated classification** of these cells, based upon a stepwise linear discriminant analysis, resulted in 80% correctly classified cells. Acceptance of confusions between successive maturation **stages** enhanced the rate of correctly **classified** cells to 100%. Among the ten most discriminating parameters, the nuclear area showed the highest correlation with the changes throughout the maturation process. The projection of the maturation sequence onto the factorial plane resulting from the canonical analysis emphasizes the existence of three phases of the maturation process, a finding that correlates well with the cytologic evolution and the biochemical and functional events during the maturation. The trajectory of cells within this factorial plane is thus regarded as a differentiation path from which a measure of the maturation could be derived.